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# Oogenesis in the Pacific Sand Dollar *Dendraster excentricus* (Eschscholtz)

by

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ABSTRACT

The fine structure of the ovarian wall and of oogenesis was examined in the irregular echinoid, Dendraster excentricus. Organelles of the oogonium, young primary oocyte, primary oocyte and the mature oocyte are compared. Emphasis is placed on the developmental changes that occur within the germ cells. Accessory cells, including follicle cells and the red pigment cells, are described.

Morphological evidence suggests that the cortical granules of this species are formed by the Golgi apparatus. Six types of cortical granules are identified and all of them contain electron dense and electron lucent material. It is proposed that the electron dense material may take part in the cortical reaction during fertilization whereas the electron lucent material may contribute to the formation of the jelly coat.

The mature Dendraster egg has an average of 130 red pigment cells evenly deposited in the jelly coat which is about 3 um thick. The red pigment cell originates from the germinal epithelium, mingles with the follicle cells and is eventually deposited in the jelly coat. The pigment cells of the mature oocyte appear to be degenerating; fewer ribosomes, and degenerating mitochondria are observed. Golgi apparati were not seen at any stage in the development of the red pigment cell.

Pigment cells are located in depressions at the cell

surface of the young oocyte. As the oocyte matures, the pigment cells are elevated into the jelly coat where degeneration occurs. The degenerating pigment cells remain situated thus until the jelly coat dissolves.

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# INTRODUCTION

Oogenesis is a complex process encompassing the origin, development and maturation of germ cells in the ovary. Echinoids in general and sea urchins in particular have received much attention in the study of ovarian structure and oogenesis. Much of this is due to the fact that sea urchins are readily available and are easily maintained in the laboratory. Moreover, sea urchin embryos have been favourite subjects for studies in developmental biology for over a hundred years (see reviews in Horstadius, 1973; Czihak, 1975; Chia and Whiteley, 1975).

Tennent, Gardiner and Smith (1931) and Tennent and Ito (1941) studied, by means of the light microscope, the organization of the ovary, the various developmental stages of oogenesis as well as a gross chemical analysis of the contents of the oocyte. In recent years, numerous studies have been done on the fine structure of the cytoplasmic organelles during sea urchin oogenesis: yolk synthesis and yolk granules (Afzelius, 1956b; Cowden, 1962; Beams and Kessel, 1963; Takashima, 1966; Kessel, 1968; Lönning, 1976), cortical granules (McCulloch, 1951; Monne and Harde, 1951; Afzelius, 1956a; Takashima, 1960; Endo, 1961; Anderson, 1968), annulate lamellae (Afzelius, 1957; Merriam, 1958; Kessel, 1964, 1967, 1968; Bal et al., 1968), nucleus and nucleolus (Afzelius, 1955, 1957; Esper, 1965; Perry, 1965, 1967; Millonig, 1966). A comprehensive re-

view of sea urchin gametogenesis was undertaken by Piatigorsky (1975) which includes references to biochemical, structural, and functional studies of sea urchins.

During oogenesis the oocytes are constantly undergoing changes not only in their size but also in the growth and distribution of the cytoplasmic elements. The production of yolk is of paramount improtance as the developing larva is dependent upon it until it is capable of obtaining its own food. In a survey of 32 species of echinoderms, Lönning (1976) has shown that the size and type of yolk granules varies greatly and appears to be directly related to the size of the egg.

Oogenesis of irregular urchins (sand dollars, heart urchins) has not been studies extensively. Unlike the regular urchins, several species of sand dollars contain red pigment cells in the jelly coat of the fully grown oocytes. Mortensen (1931, 1937) described the pigment cells as pigment granules. The development and function of the red pigment cells are unknown. Dendraster excentricus, the common Pacific sand dollar, contains red pigment granules in its jelly coat (Fow and Scheer, 1941). The oogenesis of this species has never been studied.

It is the object of this thesis 1) to gain an understanding of the fine structure of the process of oogenesis in Clypeastroidea, the irregular urchins, as it compares with the regular echinoids; 2) to elucidate the origin of the jelly coat; and 3) to add to our knowledge the struc-

ture of the red pigment cells in the jelly coat of *Dendraster excentricus*.

## METHODS AND MATERIALS

### Collection of Animals

The Pacific sand dollar, Dendraster excentricus, was collected at False Bay, San Juan Island, Washington, U.S.A. These animals aggregate into beds near the mouth of the bay and were collected monthly from May through November 1975, February, and May through August 1976. The animals were maintained in tanks with running sea water at the Friday Harbor Laboratories, University of Washington, until the ovaries were removed and fixed for later observation. The average length of the collected specimens was 8.4 cm., measuring through the mouth and anus.

### Dissection

The aboral side of the test was removed by means of a dental saw, exposing the four underlying gonads. The sex of each individual could easily be determined in ripe specimens by the colour of the gonad ( pink in the female; yellowish white in the male). Wet mounts were made to determine the sex in immature individuals.

### Electron Microscopy

The ovary was removed and fixed for two to four hours at 20°C in 2.5% glutaraldehyde in 0.4 M phosphate buffer, pH 7.6 (Millonig, 1961; Dunlap, 1966; Cloney and Florey, 1968), then washed in the same buffer for fifteen minutes.

The ovary was then cut into $1mm^3$ pieces and was post-fixed in 2% osmium tetroxide in 0.4 M phosphate buffer at 4°C for one hour. Following post-fixation, the tissue was washed with the same buffer for two fifteen minute periods, rapidly dehydrated through a graded series of ethanols and propylene oxide and embedded in Epon 812 (Luft, 1961).

One micrometer sections were examined for general orientation of the tissue. Thin sections were cut with a Sorvall Porter-Blum MT-2 ultramicrotome and those exhibiting silver-gray interference colours were collected on the matte surface of 75 or 100 mesh copper grids (Summers, 1973). The grids had been previously coated with a 2% solution of parlodion in amyl acetate (Drummond, 1950). The tissue was then stained in a saturated solution of uranyl acetate in ethanol for five minutes at 20°C (Watson, 1958). The sections were washed with distilled water, blotted dry and stained with 0.2% lead citrate for three minutes at 20°C (Reynolds, 1963). This was followed by a final wash in distilled water. The sections were examined with a Phillips 201 electron microscope.

Light Microscopy

One micrometer sections were cut with a Sorvall Porter-Blum MT-2 ultramicrotome and were stained with Richardson's stain (Richardson et al., 1960). The sections were examined with the light microscope to determine the overall structure of the ovary and its cellular components.

This preparation was most useful for comparing relative sizes of the germ cells and the accessory cells.

Ovaries were also fixed with Bouin's fixative for 72 hours or longer and treated with several changes of 70% ethanol containing a few drops of ammonia until there was no longer any trace of the picric acid in the tissue. The tissue was then immersed in 90% ethanol and two changes of 98.5% ethanol for one hour each before being transferred to 50:50 benzene:ethanol for one hour. This was followed by two 30 minute periods in benzene. The material was then infiltrated with paraffin, M.P. 56°C, at 56°-60°C for two one hour changes before it was finally embedded. Sections were cut at 5 and 7 um with an American Optical 820 microtome and were stained with Masson's trichrome (Masson,1912).

# RESULTS

## General Structure

The arborescent ovaries, four in number, are radially arranged around Aristotle's lantern. They are covered by a peritoneum which loosely attaches them to the test. The colour ranges from a creamy pink in immature individuals to a deep red in ripe specimens. The colouration is due to the presence of red pigment cells located in the egg jelly of fully grown oocytes.

Each ovary consists of a large number of tubules which interdigitate forming a network (Fig. 1). In cross section, the tubules appear either round or ovoid in shape. In the mature individual, the ovarian tubules occupy most of the internal area of the sand dollar and extend into the furthest recesses of the test. The tubules drain into the gonoduct and the mature ova are shed through the gonopore. The gonopore is located on the aboral surface of the genital plate.

## Fine Structure of the Ovarian Wall

The ovarian wall is composed of three distinct layers. The outermost layer, the peritoneum, measures 3 um at its greatest thickness and averages about 1.5 um (Fig. 2). It consists of squamous epithelial cells arranged in a single layer bordered by the basal lamina. Microvilli (Figs. 2,7) and cilia are senn on the outer surface of these cells.

The cytoplasm contains mitochondria and electron dense granules. Dense areas of clumped chromatin are seen in the nucleus.

The middle layer consists of connective tissue, smooth muscle and nervous tissue, and measures 3.5 um in width (Fig. 2). The muscle cells are arranged in a thin layer and the cells average 8-10 um in length. The cytoplasm of the cell body contains mitochondria as well as vesicles and granules. The extremities of the cell contain an increased number of vesicles and at times resemble the nerves which are located in close proximity. The nervous tissue is generally located beneath the muscle (Fig. 3); it is characterized by the presence of synaptic vesicles.

In the region of the muscle cell there are cilia embedded in the connective tissue (Fig. 2). These exhibit the typical 9 plus 2 arrangement of microtubules and are 0.2 um in diameter.

The germinal or innermost layer of the ovarian wall is quite irregular in composition (Figs. 2, 3, 4, 5), consisting of a number of cells, varying in size from very small (<1 um) to the size of the peritoneal cells (8 um) or larger. It is from this layer that the oogonia and accessory cells arise (Fig. 3).

Oogonia

The oogonium is ovoid in shape and averages 12 x 26 um in size (Figs. 8, 9). It originates at the germinal epith-

elium and juxtaposes follicular extensions at its circumference (Fig. 8). As the oogonium appears to be migrating away from the germinal epithelium, the follicular extensions interdigitate to completely surround the newly formed germ cell (Figs. 10, 11). The cytoplasm of the oogonium is homogeneous and the most evident cytoplasmic element discernible at this time is the ribosome (Fig. 8). A few small mitochondria are also present. Measuring only 0.5 um in diameter, these are aggregated at either, or occasionally both, ends of the oogonium near the nucleus (Figs. 8, 12). Golgi apparati are also found in the cytoplasm.

The nucleus is prominent in the oogonium, occupying nearly half of the area of the cell (Figs. 10, 12). An average nucleus measures 8 x 12 um. The nuclear envelope consists of two unit membranes separated by a space approximately 200 A. wide. If the membranes are indeed porous at this stage, the pores are too small to be resolved. There is a conspicuous, homogeneously staining nucleolus, 4 um in diameter, located eccentrically in the nucleus. The chromatin appears to be clumped peripherally, or as a corona (Fig. 9). Occasionally a smaller nucleolus about 1 um in diameter is also seen within the nucleus (Fig. 10).

Young Primary Oocyte

As the oogonium develops into an oocyte, a large increase in both cytoplasmic and nuclear area is noted (Table

I). This stage is marked by the onset of vitellogenesis: the appearance and growth of numerous yolk particles scattered throughout the cytoplasm (Fig. 13). At first these particles are separate and later they aggregate. These aggregate particles appear to be enclosed by a limiting membrane supplied by the Golgi apparatus and subsequently may be termed yolk granules (Fig. 14). These granules measure approximately 1 um in diameter. There is a marked increase in the number of Golgi apparati, mitochondria, free ribosomes and endoplasmic reticulum within the cell at this time.

Simultaneously with the development of the yolk granules there is the first indication of the presence of cortical granules (Fig. 13). These granules are scattered uniformly throughout the endoplasm. The cortical granules are bound by a limiting membrane and contain particles of varying electron density. In the older oocyte, six distinct and differing arrangements of the contents of cortical granules were noted. In the young oocyte, however, the cortical granules consist of an electron dense core, 0.8 um thick, surrounded by a matrix, the entire granule being enclosed by a membrane. At this time the cortical granules are small, averaging 1 um in diameter.

There are two types of non germinal cells that are associated with the oocyte at this time. The first, the follicle cell, whose structure and function will be discussed later, effectively surrounds the growing oocyte. There are

finger-like projections emanating from the follicle cells and these interdigitate so that the oocyte is encompassed by these projections. Several follicle cells may be associated with a single oocyte. The second type of non-germinal cell associated with the developing oocyte is the red pigment cell. It too will be discussed in subsequent pages. The pigment cell is located at three different sites within the ovary. It is seen amongst the follicle cells near the germinal epithelium. It is also associated with the early oocyte. In this association, it is pocketed along the oolemma. The follicle cells are surrounding the oocyte, and therefore the pigment cell is situated between the oolemma and the follicle cell. The pigment cell is also associated with the mature ovum as will be noted in more detail. The developing oocyte exhibits an assemblage of cortical granules and yolk granules. The interdigitations of the follicular extensions continue to surround the oocyte and its newly pocketed pigment cell.

Differences within the nucleus are also noted when comparing the oogonium with the young oocyte. The intact membrane of the nuclear envelope of the oogonium is perforated by nuclear pores around its circumference in the oocyte (Fig. 15). The envelope also exhibits considerable folding. The oocyte nucleolus has not changed appreciably in size or appearance from that of the oogonium; the only discernible difference being an increase in the number of smaller nucleoli. The chromatin is no longer arranged in

clumps and is dispersed uniformly throughout the nucleoplasm. It would also appear that some nuclear material has been transferred to the cytoplasm via the nuclear pores, since material of the same size and electron density was observed near the nuclear pores both in the nucleus and in the cytoplasm, as well as within the perinuclear space (Figs. 16, 17).

Evidence of micropinocytosis was noted at the plasma membrane (Figs. 18, 19).

Primary Oocyte

At this stage, the cytoplasm to nucleus size ratio has increased only slightly as compared to the ratio of increase from the oogonium to the young primary oocyte (Tables I, II). There is, however, a marked increase in the number and size of the components of the oocyte. The primary oocyte at this stage is closely associated with the follicle cells.

There are changes discernible at the oolemma. The most apparent of these changes is the appearance of numerous microvilli. These are seen along the cell surface in all stages of growth from small buds to a length of 1.5 um. The microvilli average 0.15 um in width. It is of some interest to note, however, that the microvilli do not arise along the portion of the oolemma that serves to pocket the pigment cell (Fig. 20). In every instance noted, this area of the cell surface is smoothly cupped around

the pigment cell. The pigment cell remains pocketed and closely associated with the oocyte until the jelly coat begins to form during the final phase of growth. There is still the occasional indication of micropinocytosis.

There is a large increase in the numbers of yolk granules as well as cortical granules (Fig. 20a). The average size of the yolk and cortical granules remains unchanged and measures approximately 1 um in diameter. For the most part, the granules are scattered uniformly throughout the cytoplasm. There is a region 2 um in width (Fig. 21) along the edge of the oolemma, the cortex, which does not contain any of these granules. It is also noted that the yolk granules outnumber the cortical granules by an approximate factor of two.

The Golgi apparati and the mitochondria are still very much in evidence but do not appear to have increased in either size or numbers when compared to the Golgi and the mitochondria of the young oocyte (Figs. 22a, 22b).

Annulate lamellae and their associated heavy bodies are discernible in the primary oocyte (Fig. 23). They are first observed at the nuclear membrane of the young oocyte (Fig. 24) and now they can be seen throughout the cytoplasm. The annulate lamellae are composed of two unit membranes separated by a space of approximately 200 A. These membranes resemble the nuclear membrane. They contain a central core composed of two vesicles as seen in tangential section (Fig. 24). The heavy bodies are gener-

ally surrounded by layers of annulate lamellae, but they are not completely enclosed by them. They appear about the same time as the annulate lamellae and measure 1-1.5 um in diameter and contain granules of 150 A in diameter. Occasionally larger or smaller heavy bodies may be seen within the cytoplasm.

The slight increase of the cytoplasm of the primary oocyte is accompanied by a slight increase in the size of the nucleus. The chromatin that was dispersed in the previous stage now appears granulated and is indistinguishable from the remainder of the nuclear contents. The nucleus stains less distinctly than it did previously.

The follicular extensions interdigitate and are closely associated with the oolemma and pocketed pigment cells. The oolemma, with its longer microvilli, no longer retains its contiguity with the follicle cells. Oocytes that exhibit this latter condition show other changes as well. An amorphous layer referred to as a jelly coat, begins to be deposited around the periphery of the oocyte. Once the presence of the jelly coat is noted, the pigment cells are invariably scattered throughout it and are not observed to be pocketed along the oolemma. The oocyte then enters the lumen of the ovarian tubule and the already dissociated follicle cells are left behind (Fig. 25). The internal structure of the follicle cell undergoes a dramatic change and appears to be disintegrating.

The Mature Oocyte

The mature oocyte is located within the lumen of the ovarian tubule and is separated from adjacent ova by the presence of a 1 um jelly coat surrounding each oocyte. The oocyte has a very characteristic appearance and measures 145 um in diameter in fixed sections. A membrane-bound nucleus was not observed in the mature oocyte.

The cortical granules have completed their migration through the cytoplasm and are now aligned in a single row at the oolemma. They are quite distinctive in appearance and six differing types are discernible in Dendraster excentricus (Fig. 26). Each is bound by a unit membrane. Immediately to the interior of this membrane is a substance which appears very similar to the jelly coat. The main body of the cortical granule is composed of particles of the same or differing electron densities, which may be arranged in a patterned manner. Occasionally two granules are attached together at their membranes (fig. 27a) but each granule remains distinct from the other.

The first and most numerous type of cortical granule consists of an electron dense core 0.75 um in diameter surrounded by an area 0.15-0.25 um thick, resembling the jelly coat in its stain uptake (Fig. 28a). The second type of cortical granule appears much like the first type with the addition of an inner core of material whose electron density is intermediate between the densities mentioned in the former cortical granule (Fig. 28b). A third type of cortical

granule resembles the second type, the difference between the two being that the densest part of the granule now contains two bodies midway in electron density between the outer two layers (Fig. 27b). A fourth type of cortical granule is composed mainly of an amorphous material, once again resembling the jelly coat in its stain retention, and containing three very dense bodies closely in apposition with the outer membrane of the cortical granule (Fig. 27c). The fifth type of cortical granule has the same outer layer of material as the preceeding types. The core of the granule is composed of concentric rings of material, each of a differing electron density and each of the same thickness (Fig. 27a). The remaining type of cortical granule has four areas of differing electron densities and is quite distinctive (Fig. 27d). The usual jelly-like layer is outermost. Then appear two rings bordered by a denser area and separated by a less dense but more granulated area. All of the cortical granules which were observed in Dendraster excentricus fell into one of these six categories.

Yolk platelets have reached a maximum size at this stage in the development of the Dendraster egg. The yolk granules are evenly distributed throughout the cytoplasm. They are membrane-limited and contain at least two types of particles (Fig. 14) which differ in size and density. The smaller particle is evenly distributed within the yolk granule, whereas the larger particles appear to be clustered. The largest yolk granules average 1.25 um in diam-

eter.

The microvilli at the egg's surface are considerably shorter and broader and do not appear to be as numerous as they were in the preceeding developmental stage. The largest of the microvilli atthis stage measures 0.3 x 0.2 µm.

There is an average of 130 red pigment cells embedded within the jelly coat of each Dendraster egg. The pocket of the oolemma in which the red pigment cell was contained in the earlier developmental stages is no longer present; nor indeed is there any indication of its previous location. The smooth line of the oolemma is interrupted only by the protuberances of the microvilli. The pigment cells have been elevated from the oolemma and are now embedded in the jelly coat approximately 3 µm from the egg surface (Fig. 29a).

The Red Pigment Cell

The earliest identifiable stage of the red pigment cell is found amongst the follicle cells near the germinal epithelium (Fig. 29b) and measures 4 x 5 µm in size. The nucleus is prominent and occupies a third to a half of the area of the cell. The arrangement of the chromatin is very distinctive and this, in addition to the presence of 3-4 large vacuoles, provides an easy means of distinguishing the red pigment cells from the adjacent follicle cells. The chromatin attains a thickness of 0.25 - 0.5

um around the inner circumference of the nucleus (Figs. 20, 30). This band of chromatin is complete and prevents the nucleoplasm from coming into direct contact with the nuclear envelope. There is also a large clump of chromatin in the central part of the nucleus which is variable in shape. There is no discernible nucleolus, but there are numerous densely staining bodies which may be nucleoli (Fig. 30b). The cytoplasm is quite granular and contains numerous free ribosomes. There are also small glycogen bodies which are scattered in small clusters throughout the cytoplasm. Well developed mitochondria are also present. Vacuoles, 0.5-0.75 um in diameter, are found in all pigment cells. The vacuoles contain a floccular material.

The pigment cell shows a definite association with each of the different stages of the growth of the germ cell. The oolemma of both the oogonium and the young primary oocyte is pocketed and the pigment cell is situated inside the depression that has been formed along the egg surface (Figs. 13, 20, 31a). The pigment cell of the primary oocyte remains pocketed along the oolemma (Fig. 32). Numerous microvilli cover the entire surface of the oocyte with the exception of the pocketed area in which lies the pigment cell (Fig. 20).

Recognition of the mature oocyte is facilitated by the linear arrangement of the cortical granules in the cortex of the oocyte. The pigment cells show no direct association with the mature oocyte, having been elevated from the

oocyte surface into the jelly coat. Once the pigment cell is situated in the jelly coat, about 3 μm from the oolemma, it is noted that there has been a change in the fine structure of the pigment cell (Fig. 34). The cytoplasm has deteriorated, ribosomes are few and the vacuoles have enlarged. Mitochondria are fewer and less distinct (compare Fig. 34 to Figs. 15, 20b, 20c, 30).

The red pigment cell remains in its elevated position in the jelly coat until after fertilization has occurred and the blastula has formed, at which time the jelly coat disappears as do the red pigment cells.

## The Follicle Cell

The follicle cells are numerous throughout the ovary (Fig. 34b). They are ovoid in shape and measure 3 x 7 μm in size. There are many follicle cells in association with the oogonium, and for the most part these cells do not exhibit any of the cytoplasmic extensions which are so conspicuous surrounding the young oocyte. The chromatin is rather sparse when compared to that of the red pigment cell. The arrangement of the chromatin does not appear to be consistent amongst the follicle cells. There is no evidence of a nucleolus.

The cytoplasm of the follicle cells is quite distinctive from that of the pigment cell and contains 1-3 vacuoles 1 μm in diameter. There are also several large, densely staining, spherical bodies which appear in close

proximity in the follicle cells and in oogonia which appear to be atrophic (Fig. 5). There are few mitochondria in the cytoplasm and numbers of ribosomes are sparse. The remainder of the cytoplasm is composed of small spherical granules of varying electron densities, 0.05 μm in diameter. There is evidence which suggests that the plasma membrane of the follicle cell may perforate and the small cytoplasmic granules of the follicle cells are taken up pinocytotically by the oogonium (Figs. 18, 19).

As the oogonium becomes an oocyte, there are also changes occurring with regard to the structure of the follicle cell. The most pronounced of these changes is an elongation of the follicle cell accompanied by the appearance of the long slender cytoplasmic extensions (Fig. 31b) which interdigitate to completely surround the oocyte. Also the granules within the cytoplasm of the follicle cells have become more sparse (Fig. 33a). Once the follicle cell has undergone these changes in shape, the electron dense bodies are also less numerous. Electron dense bodies similar to those in the follicle cells are found in the cytoplasm of the oocyte although no evidence was obtained of any actual transfer (Fig. 33b). Atrophied follicle cells are found in sections of the ovary that contained older oocytes and fully developed ova.

# DISCUSSION

## Ovarian Wall

The structure of the gonadal wall has been documented by a number of researchers on a variety of species representing each of the five classes of the Echinodermata: Crinoidea (Holland, 1971), Asteroidea (Tengapregasson and Delavault, 1967; Brusle, 1969), Ophuroidea (Davis, 1971), Holothuroidea (Schaxel, 1911; Atwood, 1973), Echinoidea (Wilson, 1940; Holland and Giese, 1965; Karasaki, 1965; Kawaguti, 1965; Longo and Anderson, 1969).

The terminology of the layers comprising the gonadal wall varies in the literature although the components of the gonadal wall appear to be the same. The histology of the echinoid gonadal wall was investigated by Wilson (1940). Four distinct layers were identified: an outer epithelial layer, a layer of collagen and reticular tissue, a smooth muscle layer including a second connective tissue layer and neurons and neurofibrils, and an inner germinal epithelial layer. Kawaguti (1965) describes three layers at the fine structural level: the outer coelomic epithelium, the middle connective tissue and the germinal layer. He further divides the middle layer into five component layers: the outer connective tissue, the muscle layer, the central connective tissue, the nerve plexus and the inner connective tissue.

Atwood (1973) studied the gonadal wall of the holo-

thurian Leptosynapta clarki and observed an outer coelomic layer comprised of smooth muscle processes and the "nerve cell tracts", a middle haemal sinus containing haemal fluid and coelomocytes, and an inner germinal layer. Comparing L. clarki with members of the other echinoderm classes, Atwood (1973) found that the holothurians and crinoids follow the same pattern and the three remaining classes are similar amongst themselves. These findings are in agreement with Kawaguti (1965) except that Atwood's study would seem to indicate that the nervous tissue is located in the outer epithelial layer.

The inner germinal epithelium produces the accessory cells as well as the germ cells and varies in prominence throughout the reproductive cycle. Prior to spawning it appears as a thin layer due to the fact that it has become stretched to accommodate the increased numbers and sizes of oocytes. After spawning is completed, the ovary occupies a much smaller area and its gonadal wall is at its thickest during this phase of the reproductive cycle.

Echinometra lucunter (Tennent, Gardiner and Smith, 1931) and Mespilia globulus (Tennent and Ito, 1941) deviate from the general echinoid pattern. Tennent et al. (1931) and Tennent and Ito (1941) indicate that the germ cells arise from the outer coelomic epithelium of the gonadal wall. More recent evidence drawn from electron microscopy (Kawaguti, 1965) and the present study supports the view that the germ cells arise from the germinal epithel-

ium. Perhaps the difference is attributable to species diversity. An electron microscopic analysis should be undertaken to confirm or reject the established gonadal wall structure of Echinometra and Mespilia.

Dendraster excentricus exhibits an interesting deviation from the general echinoid pattern of ovarian wall structure. Cilia are present in the middle connective layer of the wall and are embedded in the connective tissue extending to the region near the muscle. They are sparse in occurrence and may be extensions of the surface of the germinal epithelium. No direct associations were observed, however, between the cilia and any cells. Cilia also project from the inner epithelium which lines the lumen of the ovary in Nemaster rubiginosa (Holland, 1971). The cilia protrude into the lumen and not into the middle layer of the ovarian wall as is the case in Dendraster excentricus.

In most sea urchins, as in Dendraster excentricus, there is a gradient of maturity of germ cells in which the youngest, the oogonia, are closest to the germinal epithelium, and the oldest the mature eggs, are in the lumen of the ovary. Thus the oocytes do not develop synchronously.

## Yolk Synthesis and Yolk Granules

Ultrastructurally, the origin of the yolk granule has been investigated in some detail (Afzelius, 1956b; Takashima, 1966; Kessel, 1968). Afzelius (1956b) pioneered the

study of the ultastructure of a vast array of cytoplasmic elements and implicated the Golgi apparatus, or dictyosome, in yolk formation. This was carried further by Takashima (1966). He reports that the "giant vacuoles" fuse together and the primordial yolk particles appear within the amorphous ground substance. He also discusses the possibility of the Golgi and the mitochondria being involved in yolk formation. Kessel's (1968) studies on Ophioderma panamensis and Thyone briareus provide evidence that the Golgi apparatus is involved with yolk production. He suggests that the endoplasmic reticulum and, more directly, the ribosomes are responsible for the synthesis of yolk. This is in contrast to Takashima's (1966) study which states that the yolk precursor material, presumably produced by the accessory cells, is ingested by the oocyte in Hemicentrotus pulcherrimus and Pseudocentrotus depressus. Verhey and Moyer (1967a) discounted the uptake of yolk material by pinocytosis in Arbacia punctulata, Lytechinus variegatus and L. pictus. In Dendraster there was limited evidence of pinocytotic activity at the plasma membrane of the oocyte. In view of this, it seems unlikely that the limited amount of pinocytosis observed could be responsible for the presence of the yolk material in the oocyte. It seems more likely that this is synthesized within the oocyte. Once the yolk material has been formed, the Golgi apparatus stores and packages it into membrane-bound yolk granules (Kessel, 1968).

Raven (1961) suggested that the mitochondria may be involved in yolk production since the mitochondria contain many fats and the first yolk platelets are produced in areas of high mitochondrial density. There is some evidence to support this since in early oocytes mitochondria are situated in clusters at either end of the nucleus near the Golgi zone. Mitochondria, however, contribute enzymes necessary for the breakdown of fatty acids. Any lipid material that may be synthesized is incorporated into the membranes of the mitochondrion itself (Cohn, 1969; Finean et al., 1974). It would seem then that Kessel's view (1968) that it is the ribosomes that are primarily responsible for yolk production is more likely.

As the ribosome production increases, the Golgi apparati also become more numerous and the ribosomes and the endoplasmic reticulum become closely associated with the Golgi apparati. Electron microscopy has provided evidence that it is the Golgi apparatus which is responsible for storing and packaging the yolk material into yolk granules. This is evident not only in Dendraster and other echinoderms but also in other invertebrate groups such as snails (Recourt, 1961; Yasuzumi and Tanaka, 1957) and crayfish (Beams and Kessel, 1963) and some vertebrates (Ward, 1962).

## Cortical Granules and the Jelly Coat

The initial appearance of the presumptive cortical

granules in the cytoplasm is indicative that the oogonium has differentiated into an oocyte. These, along with the yolk granules, are the first characteristic organelles to appear in the early oocyte. Early electron microscopists and histologists believed the cortical granules to be an artefact of fixation. Afzelius (1956a) employed a variety of fixatives and found that the cortical granules were indeed cellular organelles and that the size of the mature cortical granules was relatively uniform. Cortical granules aligned along the cell surface of echinoderms are characteristic of mature unfertilized oocytes. Biochemical studies show that cortical granules contain sulphated mucopolysaccharides and protein (Monne and Harde, 1951; Anderson, 1968). The granules are formed in the cytoplasm and migrate to the periphery of the oocyte as the oocyte approaches maturity (Monne and Harde, 1951; McCulloch, 1952; Afzelius, 1956a; Takashima, 1960; Verhey and Moyer, 1967a; Anderson, 1968).

Two important properties of the structure and development of cortical granules have surfaced; firstly that their structure is species specific (Afzelius, 1956a) and secondly that the Golgi apparatus is responsible for their production (Anderson, 1968). The former conclusion has been upheld by the studies of Takashima (1960) and Anderson (1968). The findings of the present study reveal that the cortical granules of Dendraster excentricus are of six differing types, similar to but distinct from those of various

other species. It is conceivable that types 1-3 described earlier may be different sections of the same type of cortical granule, leaving four differing types. There is morphological evidence in Dendraster excentricus that the cortical granules are formed by the Golgi apparatus. When the cortical granules first appear in the cytoplasm of the young oocyte, they appear in areas of Golgi concentration and they were observed inside the Golgi membrane itself. The evidence would seem to indicate that the cortical granules of Dendraster are formed in the same manner as those of Arbacia (Anderson, 1968) in which the sulphated mucopolysaccharide contents of the cortical granules are secreted by the Golgi which then provides the membrane for the cortical granules by pinching the tips of the Golgi saccules. It is possible that the protein component (Monne and Harde, 1951) of the cortical granule is produced by the ribosomes and then incorporated by the Golgi into the cortical granule in a manner similar to the formation of yolk granules.

The cortical granules undergo what is commonly referred to as the cortical reaction which results in the formation of the fertilization membrane (Afzelius, 1956a; Endo, 1961). This membrane is also termed the activation calyx (Anderson, 1968). Afzelius (1956a) shows that the vitelline membrane, which is distinct from the plasma membrane, is elevated from the surface of the oocyte. This membrane forms the fertilization membrane after insemination has occurred. Endo (1961), Anderson (1968) and Sum-

mers and Hylander (1974) show that the cortical material adhering to the inner surface of the fertilization membrane accounts for the increased thickness of that structure following completion of the cortical reaction. Upon close inspection of the micrographs it is evident that it is the more electron opaque contents of the cortical granules that are adhering to the activation calyx.

Before fertilization occurs, the mature cortical granules are aligned in a single row along the oolemma. At this time there is considerable material inside the granules which is less electron dense, is composed of sulphated mucopolysaccharide (Afzelius, 1956a) and has the same staining properties as the jelly coat. This less electron dense material is not observed along with the electron opaque material after fertilization. It follows then that the less electron dense material may be released from the cortical granules before fertilization takes place, thus contributing to the formation of the jelly coat. Takashima (1960) reports that the peripheral portion of the cortical granules in fully ripe oocytes shows "breakdown or obscueness". It is during the time that the cortical granules are aligned along the oolemma that the jelly coat attains its greatest diameter. The cortical granules are not responsible for the formation of the entire jelly coat since the jelly coat appears before the cortical granules have completed their migration to the oolemma.

Holland (1971) reports that "there is no morphological evidence that the accessory cells take part in the formation of the jelly layer" in Nemaster rubiginosa and states that the jelly coat precursors are the contents of ovoid granules which appear in the peripheral cytoplasm just prior to the appearance of the jelly coat. This would serve then to provide the early jelly coat which is further augmented by the less electron dense material of the cortical granules. In D. excentricus it is possible that these events could occur as described resulting in the formation of the jelly coat.

Annulate Lamellae

There has been some controversy regarding the origin and possible function of the annulate lamellae. Merriam (1958) and Kessel (1964, 1968) presented the view that the annulate lamellae are formed by a fusion of vesicles formed by the nuclear membrane. Kessel (1964) was more specific and said that the origin of annulate lamellae was a blebbing process of the outer layer of the nuclear envelope. Bal et al. (1968) confirmed the nuclear origin of the membrane system but found that the actual formation of the lamellae was due to a folding of the nuclear envelope rather than a fusion of the vesicles formed by the envelope. The present findings indicate that there is extensive folding of the nuclear membrane in the region of the annulate lamellae. There was little or no evidence

of vesicles being formed by the nuclear envelope. It would appear then that the annulate lamellae of Dendraster excentricus are formed in the same manner as those of Arbacia punctulata (Bal et al., 1968).

The results of this study disagree in part with Merriam (1958) who also studied the annulate lamellae in Dendraster excentricus. The actual structure of the lamellae - paired membranes with annuli in regular hexagonal arrangement - was confirmed. The discrepancy arises when investigating the time of the appearance of the lamellae in the development of the oocyte. Merriam (1958) reports that the annulate lamellae are seen in the mature ovum usually after the disappearance of the germinal vesicle. In the present study the annulate lamellae were found in the primary oocyte during vitellogenesis. Bal et al., (1968) found that the annulate lamellae of Arbacia punctulata were absent during vitellogenesis and present at other stages of development of the oocyte. Afzelius (1957) indicated that the annulate lamellae are associated with large amounts of ribonucleoprotein probably derived from the nucleolar material of the nucleus. According to Kessel (1968), the annulate lamellae play a role in protein synthesis and may perhaps have some sort of transcriber function. In view of these findings then, one would expect to find annulate lamellae present during vitellogenesis and during the time that the cell is active in protein synthesis. Such is the case in the present study

of Dendraster excentricus. Annulate lamellae are found in both invertebrate and vertebrate tissue (Raven, 1961). They are most prominent in cell types undergoing rapid growth and differentiation (Afzelius, 1955, 1957; Kessel, 1964) and therefore one would expect to find these structures in Dendraster oocytes.

Nucleus and Nucleolus

Since it is the nucleus that contains the genetic information and is responsible for the production of RNA, the events occurring in the nucleus and nucleolus are of importance in the development of the egg cell. The time at which the changes take place should also be noted in relationship to the overall development of the cell.

The nuclear membrane of the Dendraster oogonium is non-porous. This was also reported in Mespilia globulus (Tennent and Ito, 1941). The nuclear pores form during the primary oocyte stage (Verhey and Moyer, 1967a; Piatigorsky, 1975) after which the passage of ribosome-like particles and nucleolar material into the cytoplasm appears to take place.

During the previtellogenic growth of the oocyte, the numbers of ribosomes in the cytoplasm increase several hundred times (Verhey and Moyer, 1967a; Millonig et al., 1968). At the same time, the nucleus and nucleolus are undergoing increases in size (Esper, 1965; Holland and Giese, 1965). One would expect to find the transfer of

information to the cytoplasm at this stage of rapid growth and it is thought that the ribosome-like particles of the nucleolus are transferred to the cytoplasm (Tennent and Ito, 1941; Millonig et al., 1968). The extrusion of nucleolar material into the cytoplasm is common in sponges, hydroids, platyhelminths, annelids, molluscs, arthropods and vertebrates (Raven, 1961). Millonig et al. (1968), however, did not observe the passage of any material from the nucleus to the cytoplasm in Arbacia lixula and Paracentrotus lividus. They reported a dense homogeneous material within the nuclear pores which presumably blocked the passage of material from the nucleus to the cytoplasm. In Dendraster excentricus there is evidence of the transfer of nucleolar material through the nuclear pores into the cytoplasm. This occurs in the previtellogenic stages when the oocyte is undergoing rapid growth which also corresponds with the hundredfold increase in the numbers of ribosomes. In view of this, it is interesting to note that the primary function of the nucleolus is to synthesize ribosomal RNA (Perry, 1965, 1967; Esper, 1965). It would appear then that the transfer of nucleolar material to the cytoplasm has a direct linkage with the transfer of ribosomal RNA.

As the oocyte approaches maturity, the nucleus and nucleolus undergo physical changes culminating in their disappearance. Towards the end of vitellogenesis, the major nucleolus becomes vacuolated and disappears (Millon-

ig et al., 1968). At this time the nucleolus undergoes changes in volume and stainability until it disappears completely at meiosis (Afzelius, 1957; Esper, 1965). The maturation divisions of the sea urchin egg differ from those of starfishes and most other invertebrates since the maturation divisions are completed before fertilization (Afzelius, 1955).

The Red Pigment Cell

The red pigment cell, located in the jelly coat of the mature oocyte, is characteristic of Dendraster excentricus. Summers and Hylander (1974) published the first account of the presence of red pigment cells in the jelly coat of a sand dollar, Echinarachnius parma. These were previously termed pigment granules in Dendraster excentricus (Fox and Scheer, 1941).

The course of the red pigment cell from its inception until its appearance in the jelly coat is one of marked migration. The red pigment cell is a non-germinal cell produced by the germinal epithelium. In its early stages the pigment cell is closely associated with the follicle cells. It then begins its migration and comes to lie near the periphery of a young oocyte or oogonium. The follicular association with the red pigment cell is still present and the pigment cell is wedged against the oolemma of the oogonium or young oocyte while the follicular extensions lie adjacent to the free surface of the red pigment cell.

As the oocyte enters its developing phase (pre-vitellogenic), the pigment cell becomes pocketed along the oolemma. The pocket is simply an indentation of the oolemma. Since there does not appear to be any growth or change within the red pigment cell at this time, it would seem that the red pigment cell is pocketed in readiness for its contribution to the mature oocyte. Further growth of the oocyte leads to its maturation phase, during which the red pigment cell is elevated into the jelly coat. The pigment cell appears to have entered a slow phase of degeneration which will culminate in the disappearance of the pigment cell upon the dissolution of the jelly coat.

The function of these pigment cells is unknown. In view of the fact that there is an average of 130 red pigment cells in the jelly coat of each Dendraster egg, one would assume that they must fulfill a role in oogenesis. Their marked migration and change in internal structure is consistent. Prior to degeneration the pocketed pigment cell is closely associated with the growing oocyte and may contribute to the oocyte in some manner which remains undetermined. It seems unlikely that the pigment cell would have an active function in its degenerating phase.

## The Follicle Cell

Follicle cells are important to the process of oogenesis in Dendraster excentricus as they are in the development of many other invertebrates including members of the

Porifera, Anthozoa, Hirudinea, Gastropoda, Cephalopoda, Insecta, Ascidia (Raven, 1961). Raven (1961) distinguishes between follicle cells and nurse cells. The nurse cells tend to be much larger than the follicle cells and are purported to be active in nourishing the growing oocyte (Raven, 1961). The nurse cells tend not to surround the oocyte completely in contrast to the follicle cells. Most insects have both follicle cells and nurse cells in the ovary (Raven, 1961).

The follicle cells completely surround the growing oocyte and may (Holland and Giese, 1965; Chatlynne, 1969) or may not (Holland and Giese, 1965; Verhey and Moyer, 1967a; Chatlynne, 1969) contribute significantly to the growth of the oocyte. There is species specificity with regard to the amount and type of material transferred to the oocyte. The follicle cells of Arbacia punctulata, Lytechinus variegatus and L. pictus do not contain glycogen (Verhey and Moyer, 1967b) in contrast to those of Strongylocentrotus purpuratus which do (Chatlynne, 1969). The specific function of the follicle cells in oocyte growth remains unclear (Piatigorsky, 1975).

Dendraster excentricus is found to possess follicle cells as do all other echinoderms thus far documented (Raven, 1961). Since histochemical studies were not undertaken it is not possible to determine precisely the contents of the follicle cells. It may be surmised from the staining properties of the follicle cells that they

do contain glycogen and fat globules. The membrane of the follicle cell breaks down near the oocyte membrane shortly before pinocytosis occurs. The extrusion of nutritive substances in Hemicentrotus pulcherrimus and Heliocidaris crassispina into the intercellular space corresponds with the breakdown of the follicle cells (Takashima and Takashima, 1965). There are "seasonal" changes of the follicle cells which follow the progress of egg development (Takashima and Takashima, 1965).

From studying the electronmicrographs it appears that the pinocytotic material taken up by the oocyte was originally present in the follicle cells. When one considers the limited amount of material taken up pinocytotically by the oocyte as well as the tremendous increase in size and complexity undergone by the germ cell, it would seem that pinocytosis contributes minimally to oocyte development. Indeed isolated oocytes of Lytechinus pictus are reported to be active in RNA and protein synthesis (Piatigorsky et al., 1967).

In addition to the nutritive function of the follicle cells, Hirai et al. (1973) show in Asterina pectinifera that the follicle cells are instrumental in stimulating the oocytes to mature. Gonad stimulating Substance (GSS) is released from the nervous tissue into the coelomic cavity (Kanatani, 1969) which stimulates the follicle cells to produce a maturation inducing substance (MIS) (Hirai et al., 1973). The fully grown oocytes respond to the

presence of MIS and undergo maturation divisions and spawning. It was emphasized (Hirai et al., 1973) that only the follicle cells surrounding fully grown oocytes can respond to GSS in producing MIS and that only fully grown oocytes will respond to MIS.

## SUMMARY

The ovary of Dendraster excentricus develops asynchronously and each of its developing components (oogonium, young primary oocyte, primary oocyte, mature oocyte, follicle cell and red pigment cell) is distinctive in its ultrastructure. During oogenesis there is a marked migration of the germ cells within the ovary and of the red pigment cells both within the ovary and in association with the oocyte.

With the exception of Echinometra lucunter (Tennent et al., 1931) and Mespilia globulus (Tennent and Ito, 1941), the oocyte in this member of the Clypeastroidea proceeds as within the regular sea urchins. The detailed ultrastructure of each stage is described and compared to regular echinoids whose oogenesis has been studied.

The structure of the jelly coat is postulated; however, its origin has not been established. In order to do so, one could do an autoradiographic study and label the protein component of the jelly coat with tritiated leucine.

The origin, development and structure of the red pigment cells present in the jelly coat of Dendraster are documented. This represents an important step in the study of oogenesis of the Pacific sand dollar since each animal contributes approximately 130 red pigment cells to the jelly coat of each egg. The pigment cells were heretofore undescribed.

TABLE I

Size Differentiation of Early Growth Stages
of the egg of Dendraster excentricus

| Developmental Stage | Cytoplasm Average Cross Sectional Area (um) | Nucleus Average Cross Sectional Area (um) |
|---|---|---|
| Oogonium | 300 | 100 |
| Young primary oocyte | 6400 | 1000 |
| Primary oocyte | 6900 | 1400 |

TABLE II

Growth Ratios of Early Growth Stages
of the egg of Dendraster excentricus

| Area of Cell | Growth Stage* | Growth Ratio |
|---|---|---|
| Cytoplasm | AB<br>BC | 6.89 |
| Nucleus | AB<br>BC | 3.67 |

*
A- Oogonium
B- Young primary oocyte
C- Primary oocyte

Figure 1. Photograph of the ovary of Dendraster excentricus illustrating the tubular nature of the ovary. O, oocyte; T, ovarian tubules. 50x

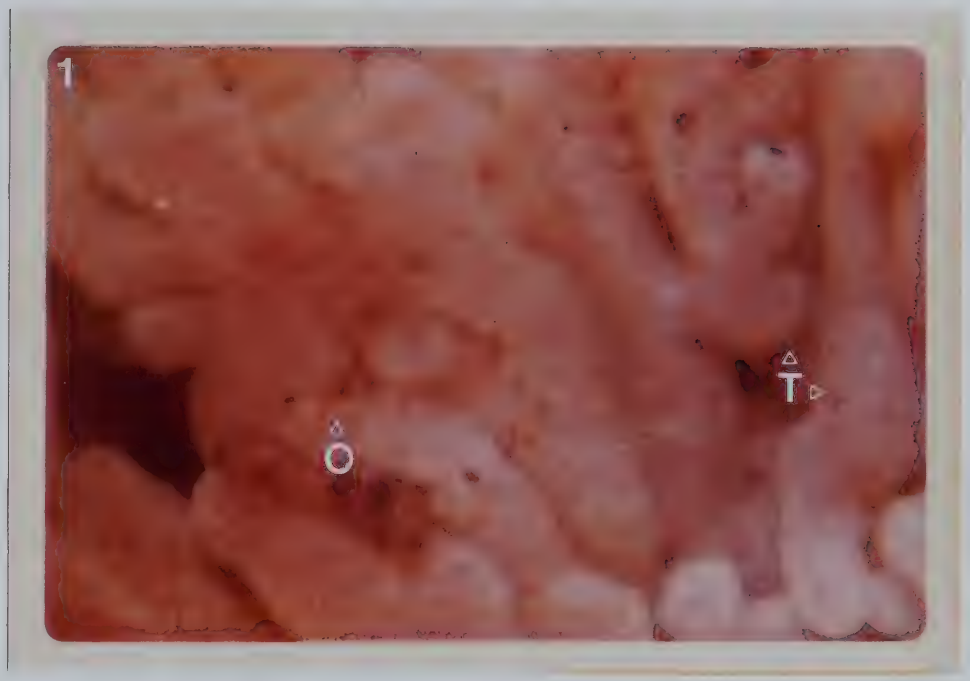
1
T
O

Figure 2. Ovarian wall. Note cilium in middle layer. c, cilium; ct, connective tissue; e, epithelial cell; g, germinal epithelium; mi, microvilli; sm, smooth muscle. (All scale bars represent 1 um unless otherwise indicated).

Figure 3. Ovarian wall showing detail of germinal epithelium. b, basal lamina; g, germinal epithelium; nt, nervous tissue.


2
mi
e
ct
sm
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g

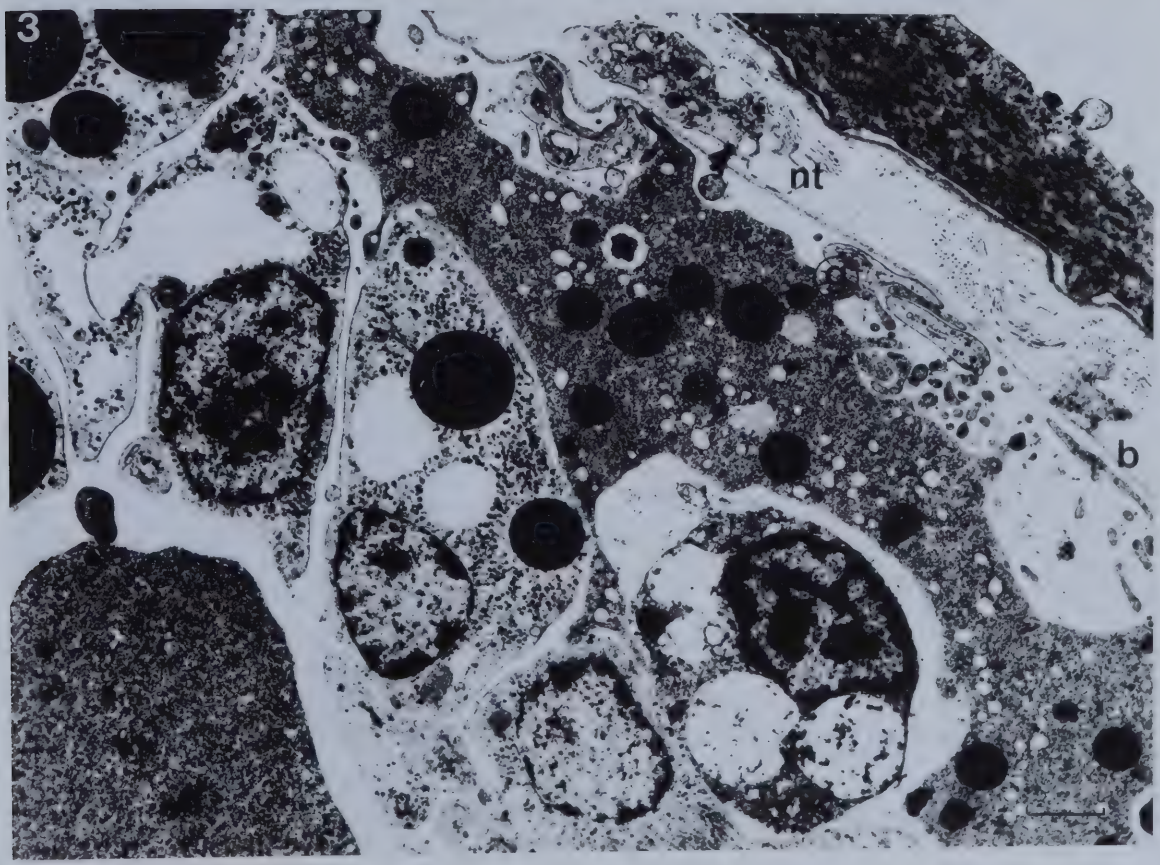
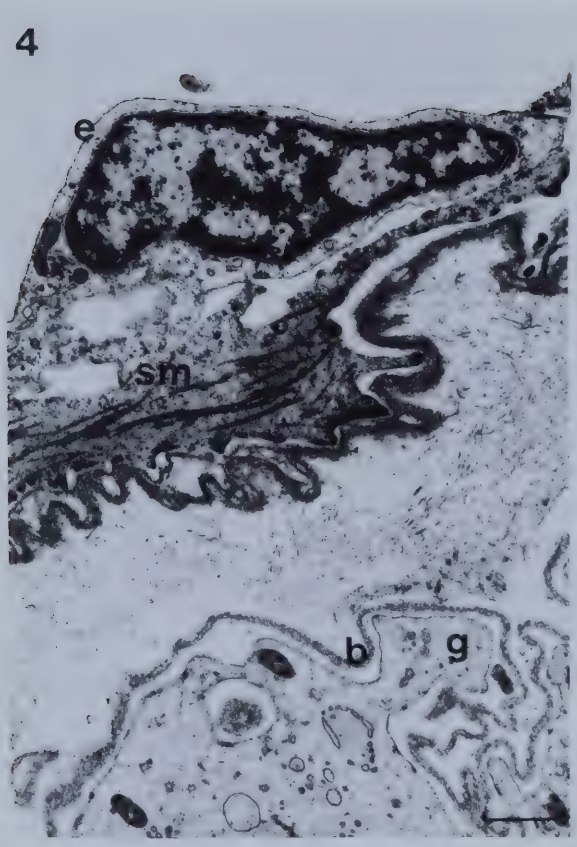

3
nt
b

Figure 4. Ovarian wall in contracted state. b, basal lamina; e, epithelial cell; g, germinal epithelium; sm, smooth muscle.

Figure 5. Ovarian wall showing irregular nature of the germinal epithelium. b, basal lamina; g, germinal epithelium.

Figure 6. Ovarian wall showing detail of the middle connective layers. b, basal lamina; e, epithelial cell; g, germinal epithelium; sm, smooth muscle.

Figure 7. Ovarian wall illustrating three distinct layers, the outer epithelial layer, the middle connective layer and the inner germinal layer. b, basal lamina; e, epithelial cell; g, germinal epithelium; mi, microvilli; sm, smooth muscle.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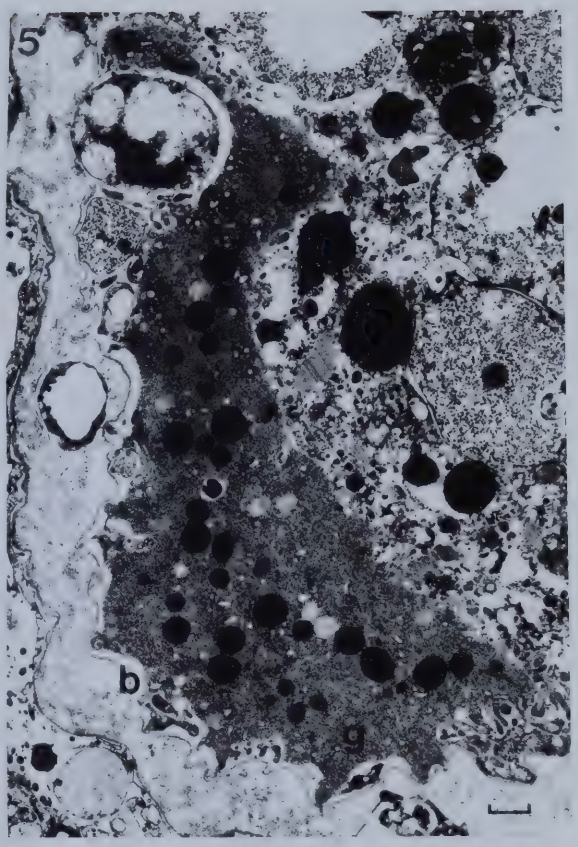
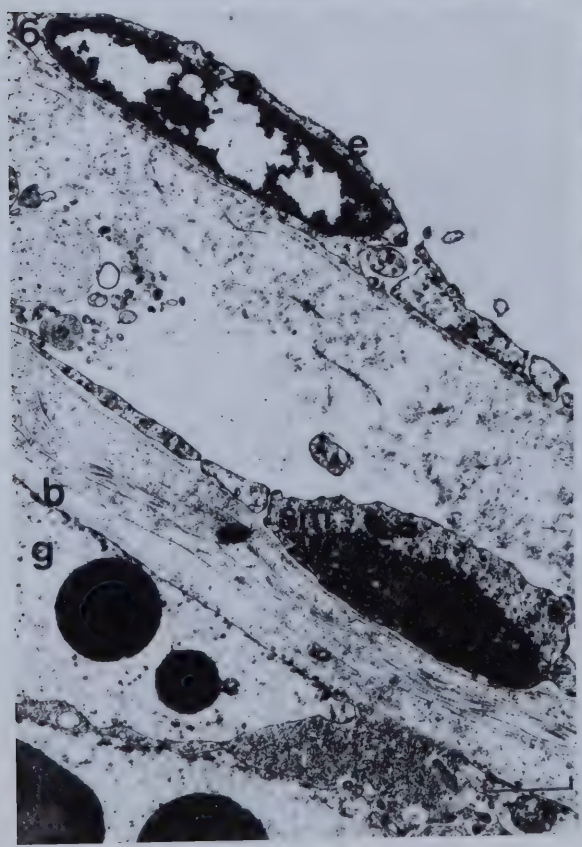
e
sm
b
g


5
b
g


6
e
b
g
sm

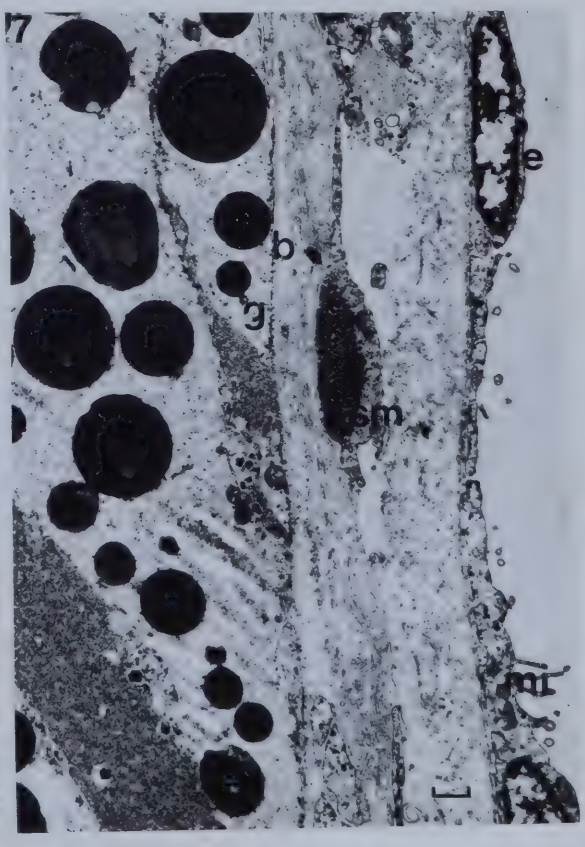
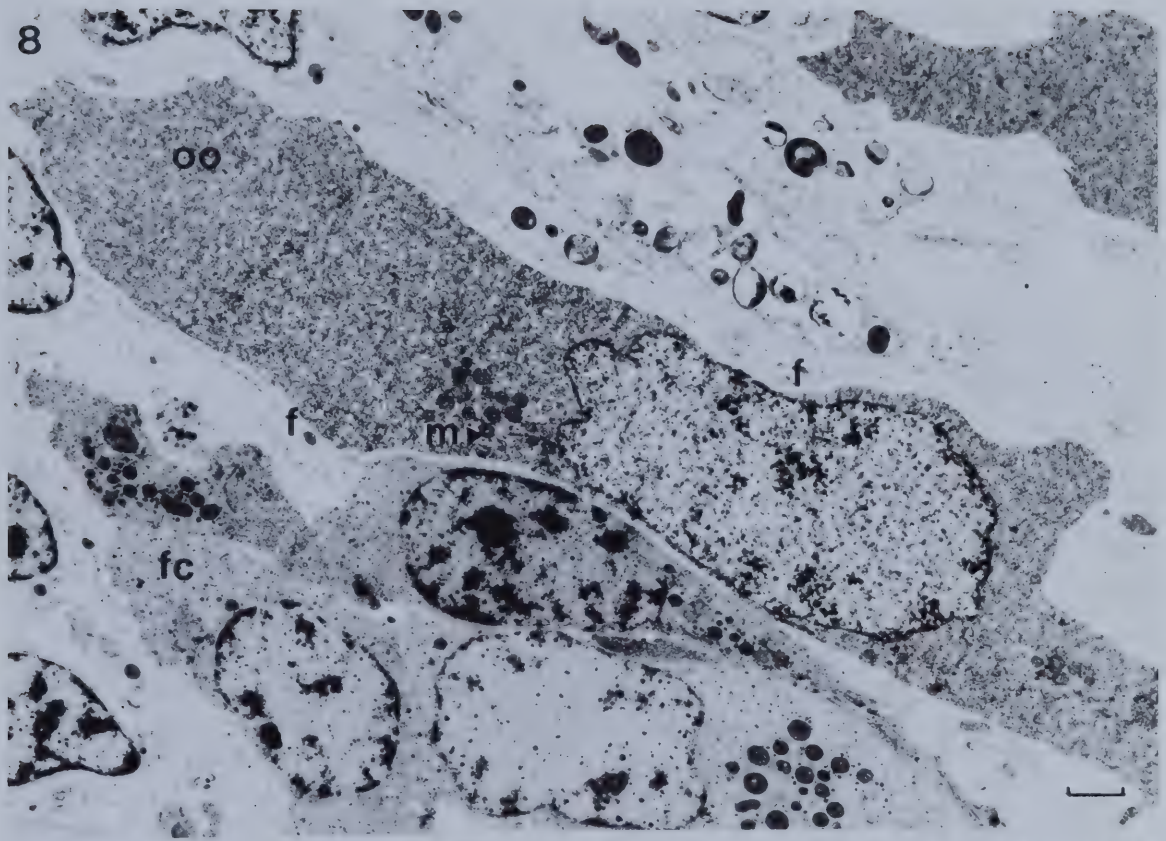

7
e
b
g
sm
mi

Figure 8. Oogonium and follicle cells. Note the clumping of the mitochondria at opposite sides of the nucleus. f, follicular extensions; fc, follicle cells; m, mitochondria; oo, oogonium.

Figure 9. Oogonia and follicle cells arising from the germinal epithelium. b, basal lamina; fc, follicle cells; g, germinal epithelium; m, mitochondria; oo, oogonium; sm, smooth muscle.


8
oo
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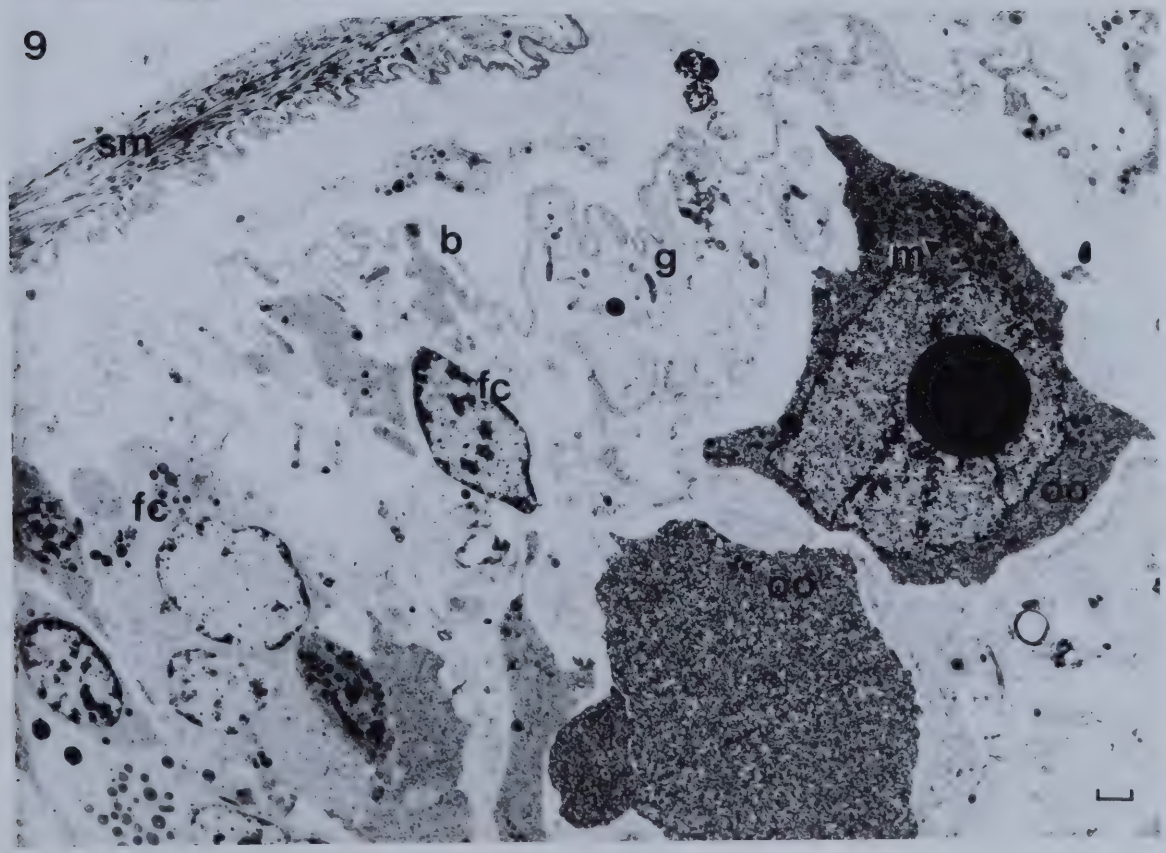
fc


9
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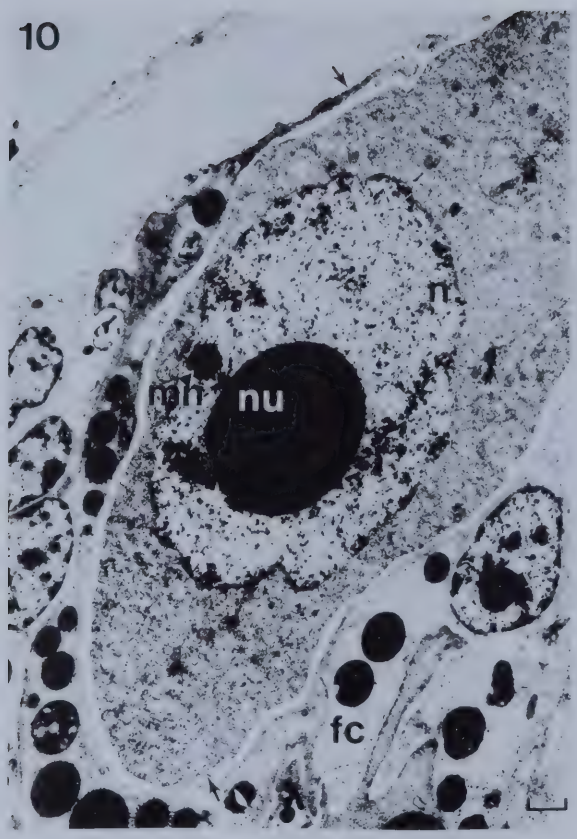
oo

Figure 10. Oogonia near the germinal epithelium. Note the follicular extensions (arrows). fc, follicle cells; mn, minor nucleolus; n, nucleus; nu, nucleolus.

Figure 11. Oogonium having migrated away from the germinal epithelium. Note the follicle cells and follicular extensions (arrows) surrounding the oogonium. fc, follicle cells.

Figure 12. A typical oogonium showing well defined nucleus and nucleolus. Note the clustered mitochondria. m, mitochondria; n, nucleus; nu, nucleolus.

Figure 13. Young primary oocyte in early vitellogenic stage. cg, cortical granule; y, yolk granule.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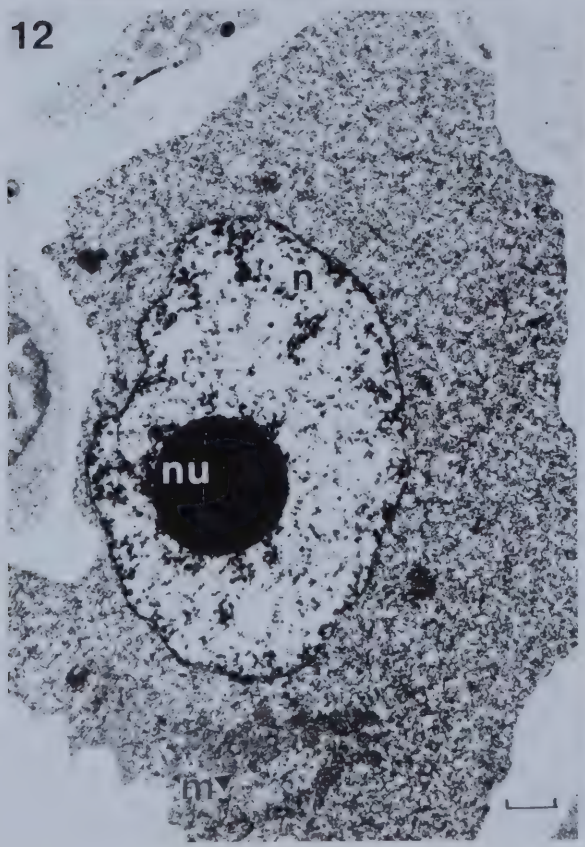
fc


11
fc
fc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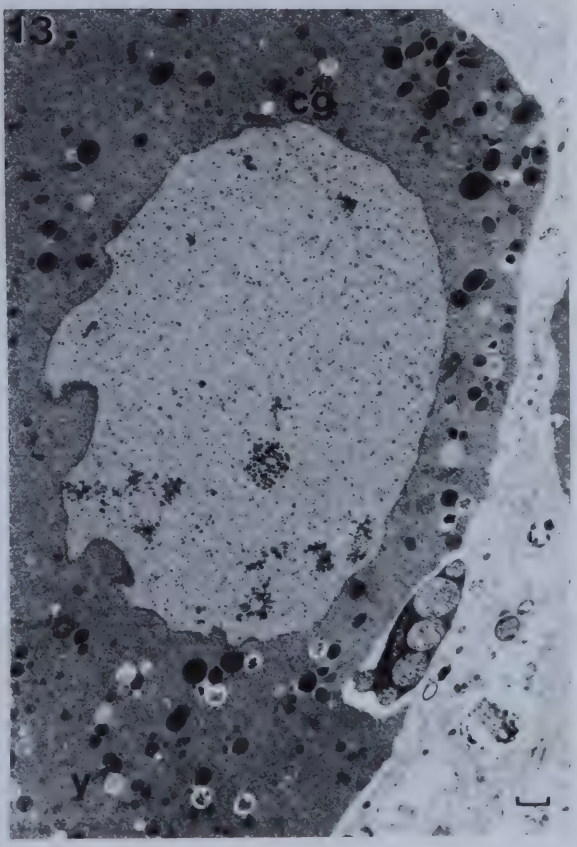
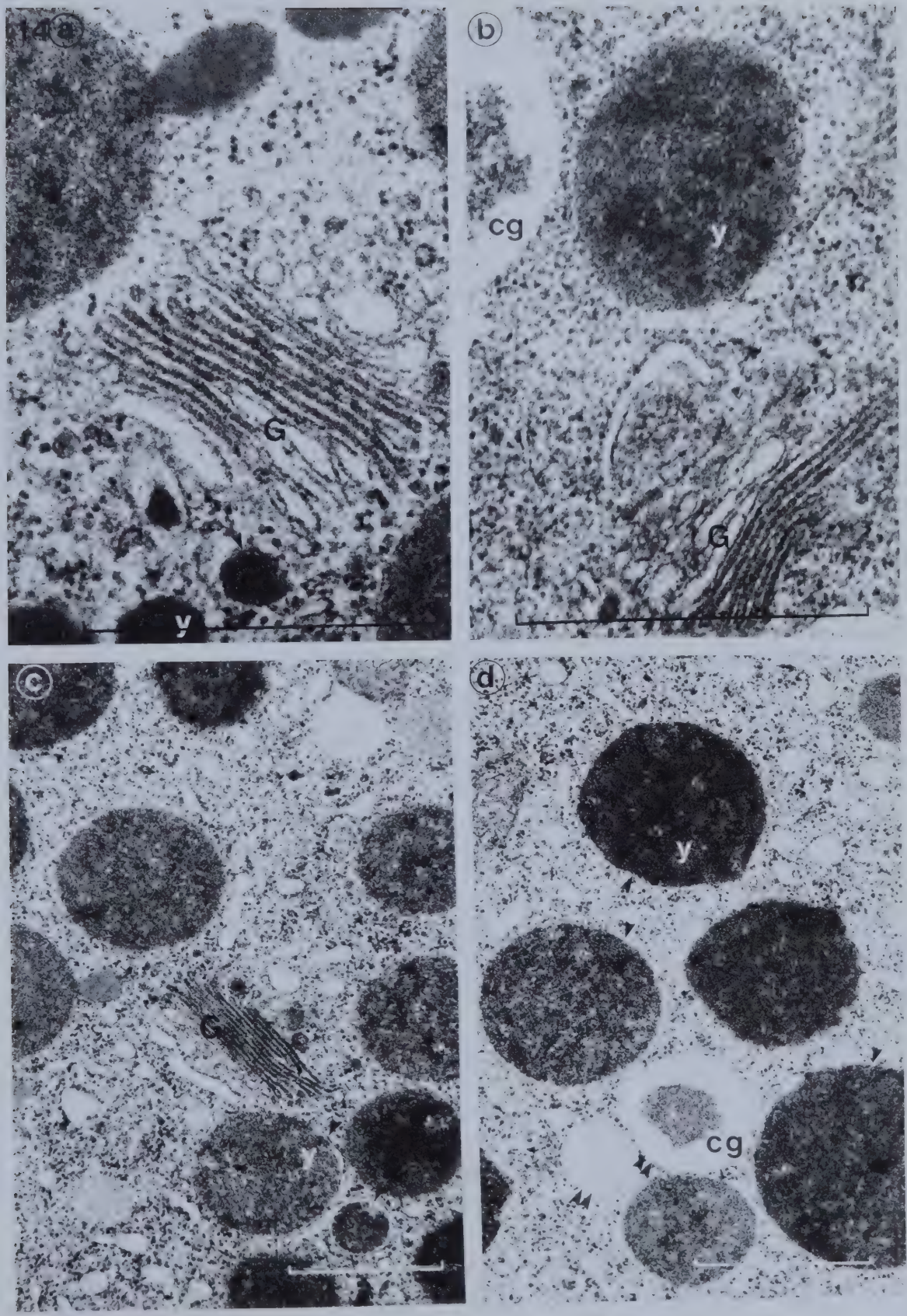

13
cg
y

Figure 14. Detail of Golgi apparatus and its involvement in the formation of cortical granules and yolk granules. a) membrane-limited yolk granules near Golgi region. Arrows indicate membrane which could have been produced by the Golgi apparatus. b) yolk granule and cortical granule, both membrane bound in region of Golgi. c) low magnification micrograph showing the Golgi apparatus incorporating yolk particles into yolk granules. d) newly formed yolk and cortical granules showing limiting membrane. c, cortical granule; G, Golgi apparatus; y, yolk granule; ►, membrane of yolk granule; ▶, membrane of cortical granule.


14 a
G
y
b
cg
y
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y
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y
cg

Figure 15. Electronmicrograph of a young primary oocyte showing the porous nature of the nuclear membrane (arrows).

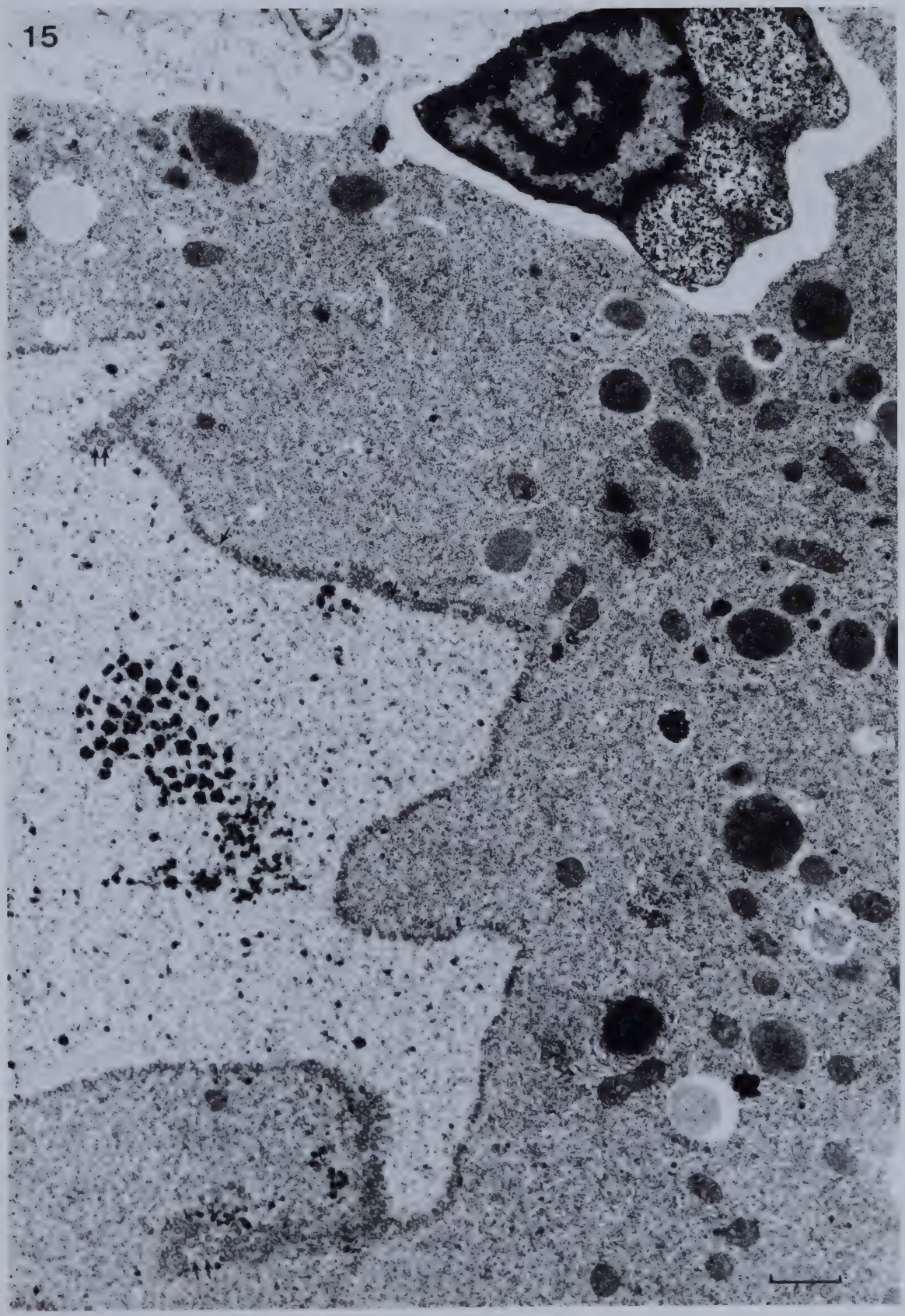
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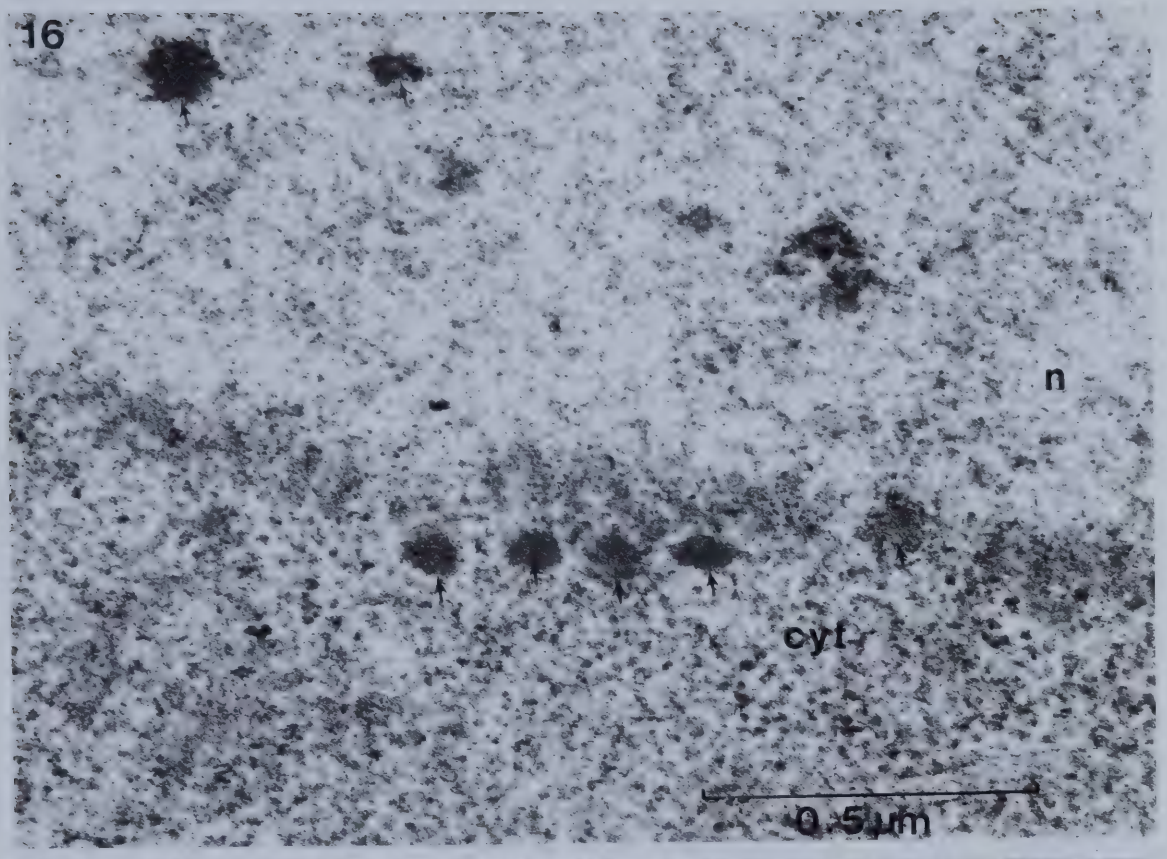
Figure 16. Nucleus and cytoplasm of young primary oocyte containing bodies of the same electron density (arrows).

Figure 17. Electron dense bodies present in nucleus, nuclear pores and cytoplasm (arrows).


16
n
cyt
0.5 µm

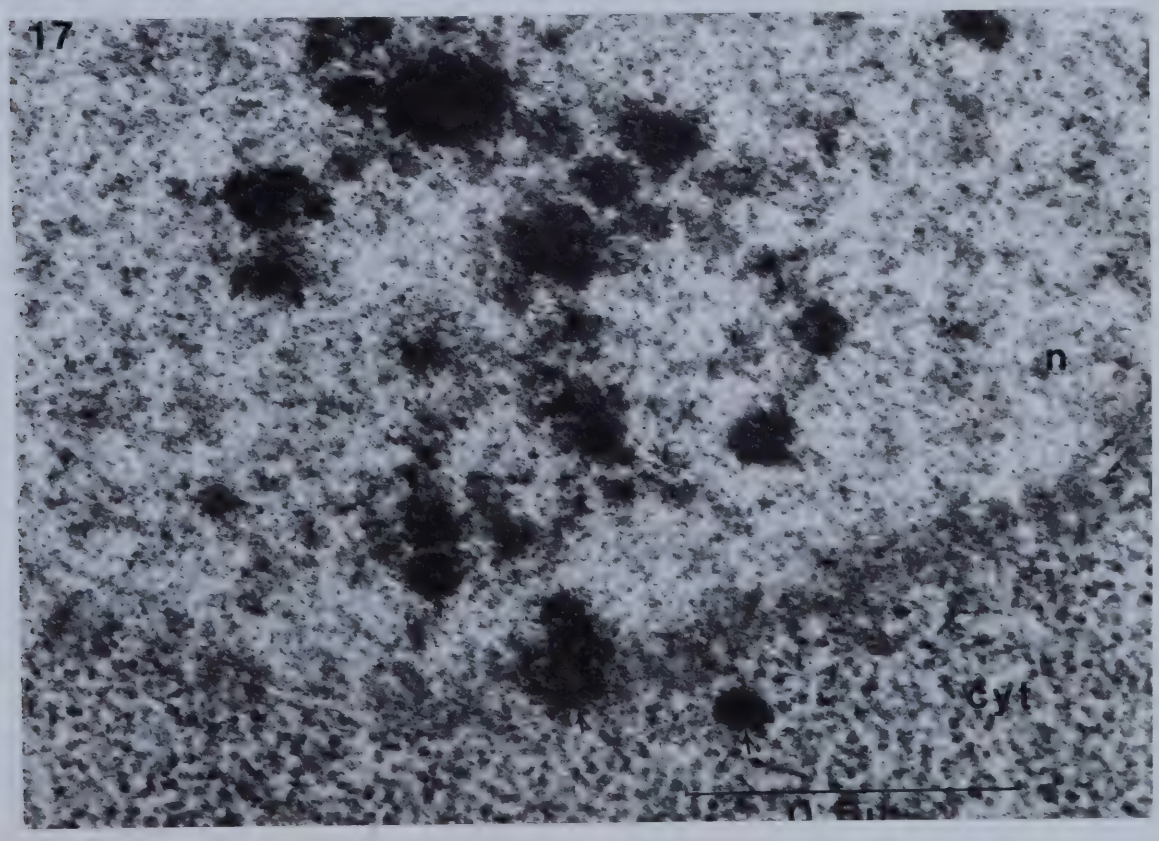

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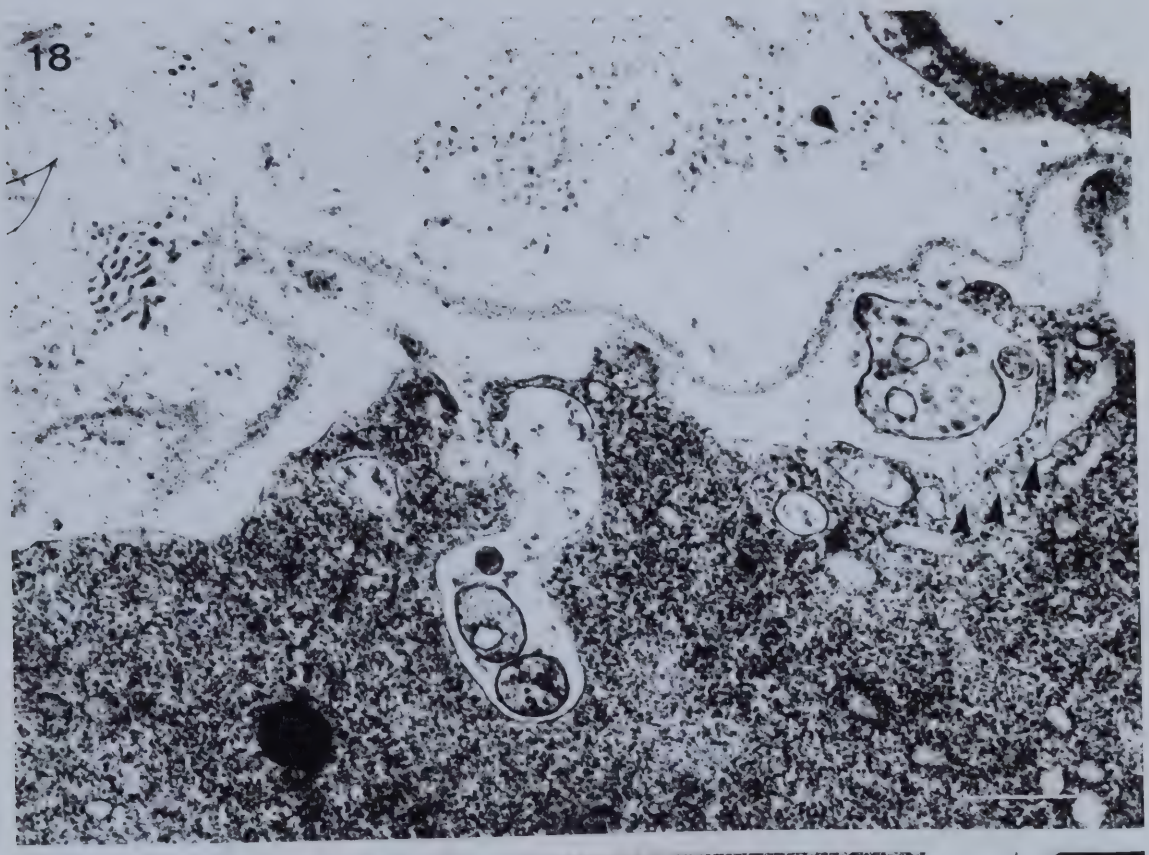
Figure 18. Cell surface of the young primary oocyte showing evidence of micropinocytosis (arrows).

Figure 19. Micropinocytosis at cell surface (arrow). Follicular material appears to be taken up by the oocyte (large arrow).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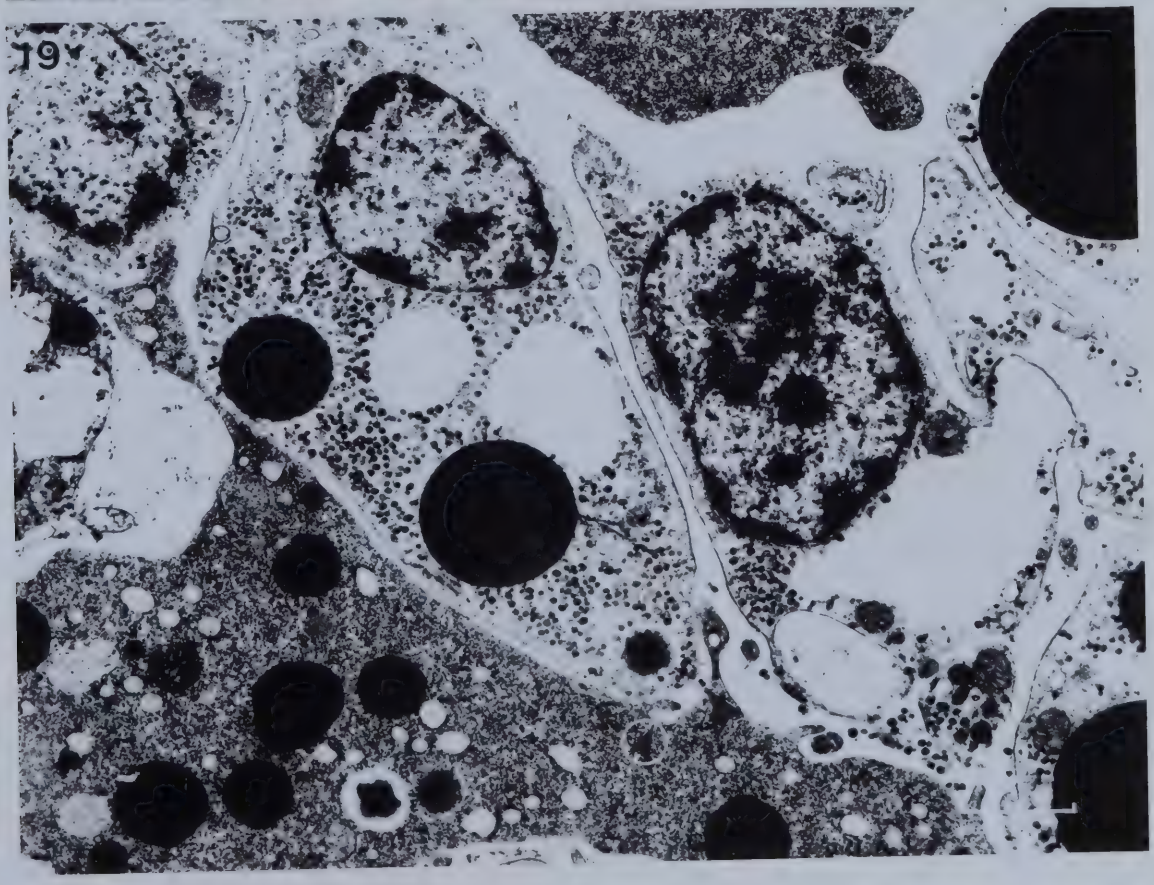
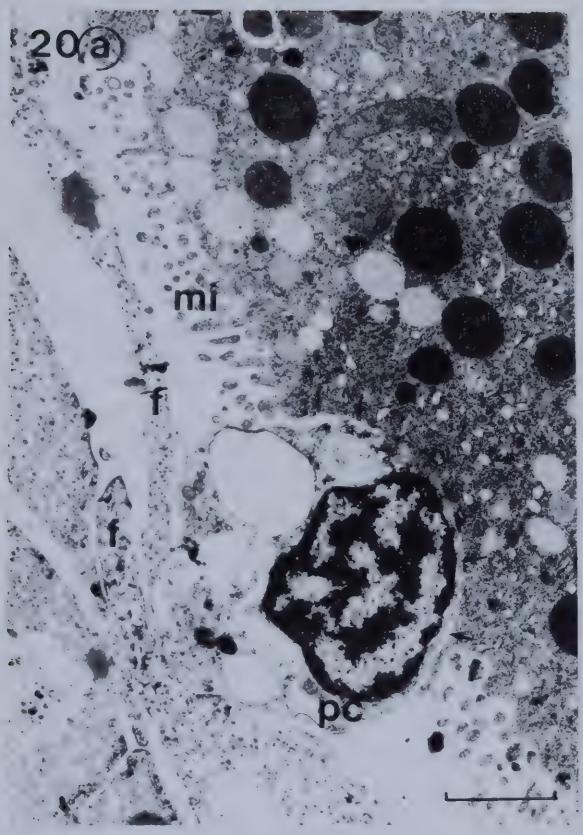

19

Figure 20. Pigment cell associations with the primary oocyte. a) follicular extensions surround the oocyte and its pocketed pigment cell. Note the absence of microvilli in the pocket of the pigment cell(arrows). b) microvilli are absent from the oolemma in the region of the pigment cells. Follicular associations are not pronounced as in a) and c). c) the cytoplasm of the pigment cell appears to be less distorted than in a). f, follicular extension; mi, microvilli; pc, pigment cell.

Figure 21. Primary oocyte showing cortical region which is free from mitochondria, yolk granules and cortical granules. cx, cortex.


20a
mi
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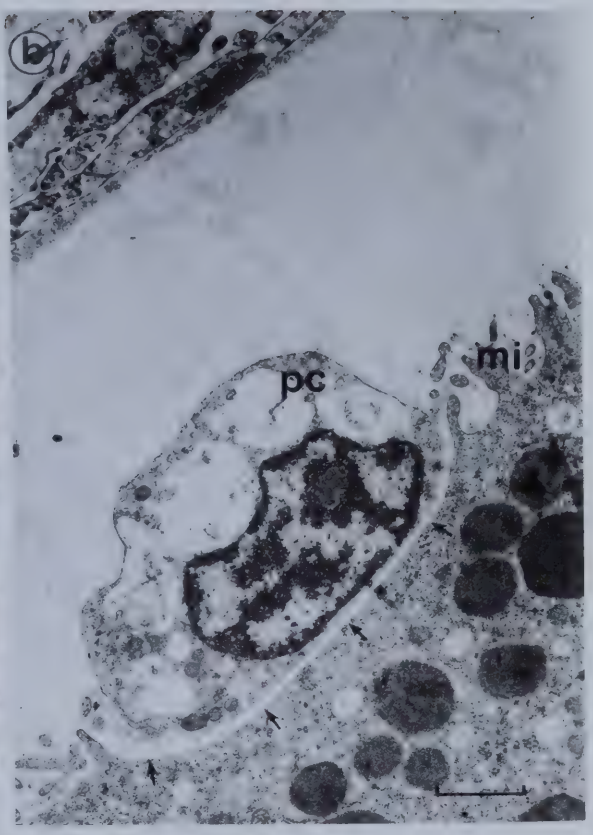
f
pc


b
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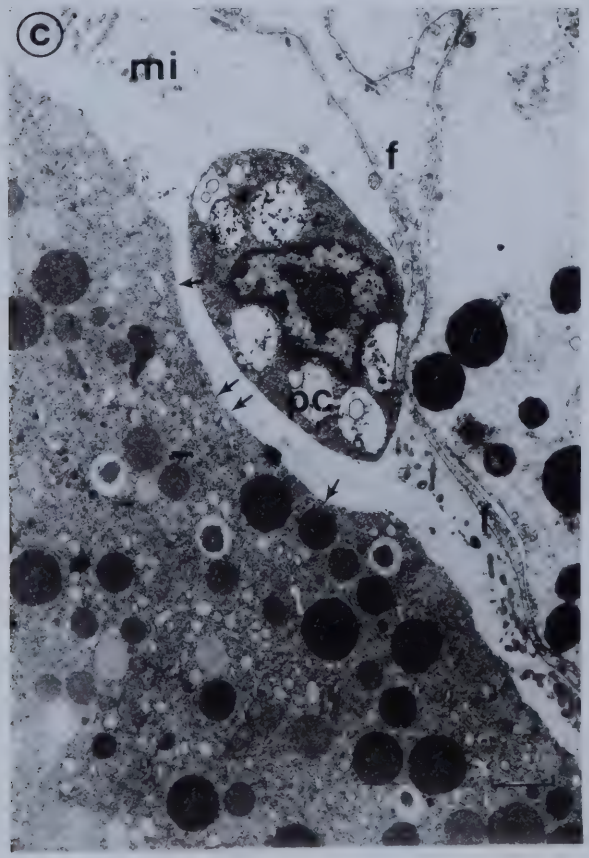
pc


c
mi
f
pc
f

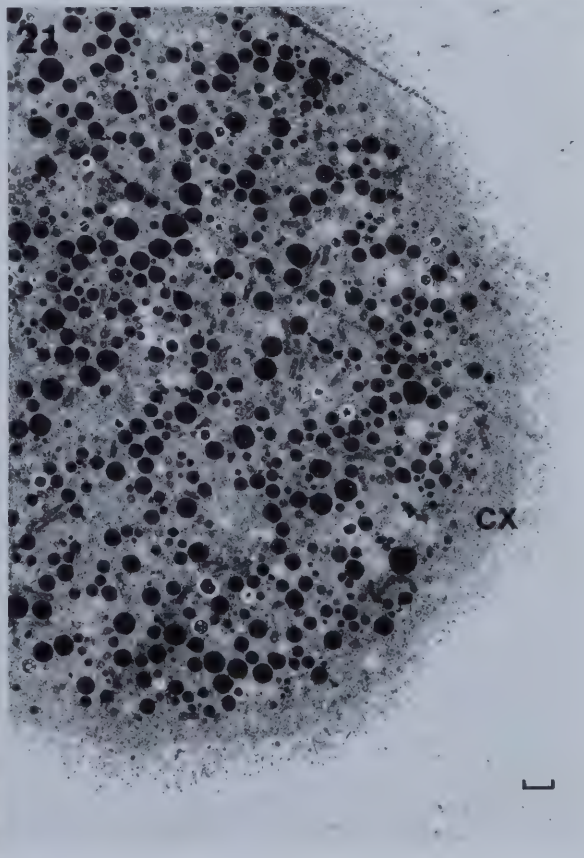
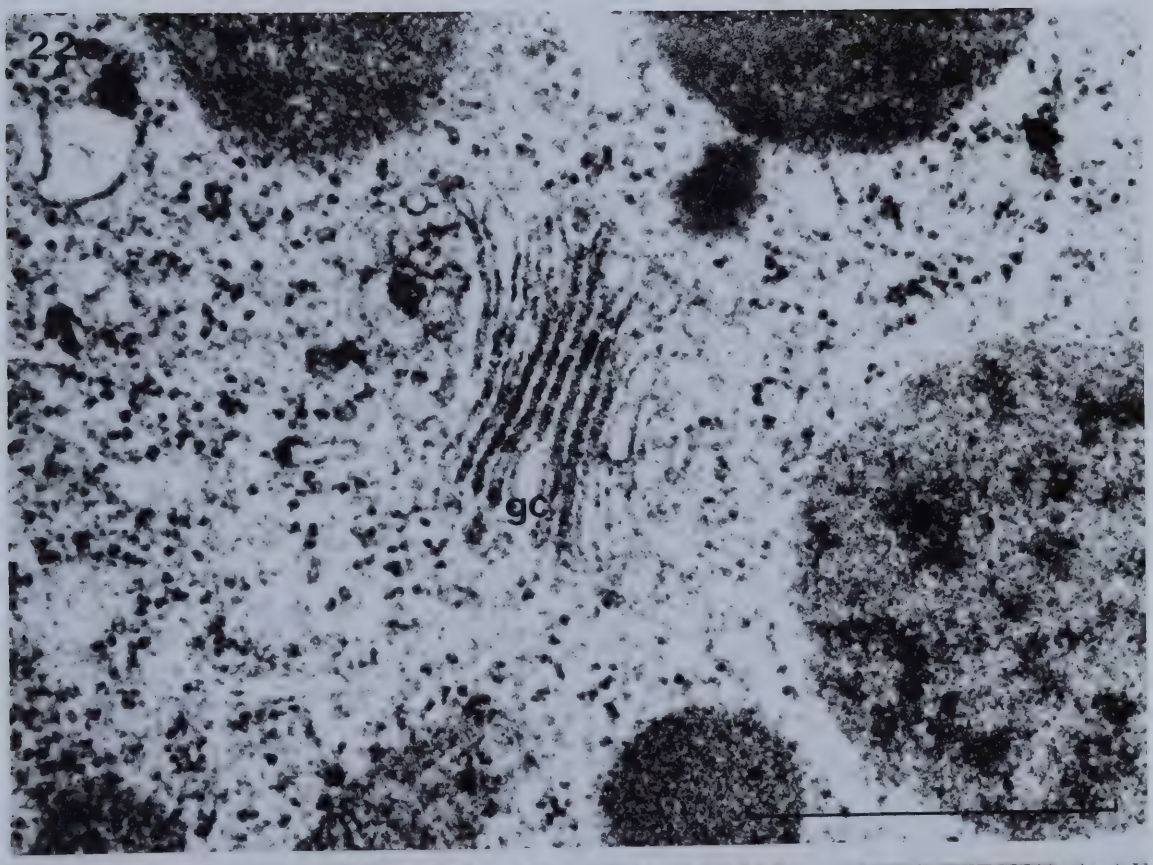

21
cx

Figure 22. Cellular organelles of the primary oocyte. a) Golgi and b) mitochondria of the primary oocyte appear unchanged from those of the young primary oocyte.


22
gc

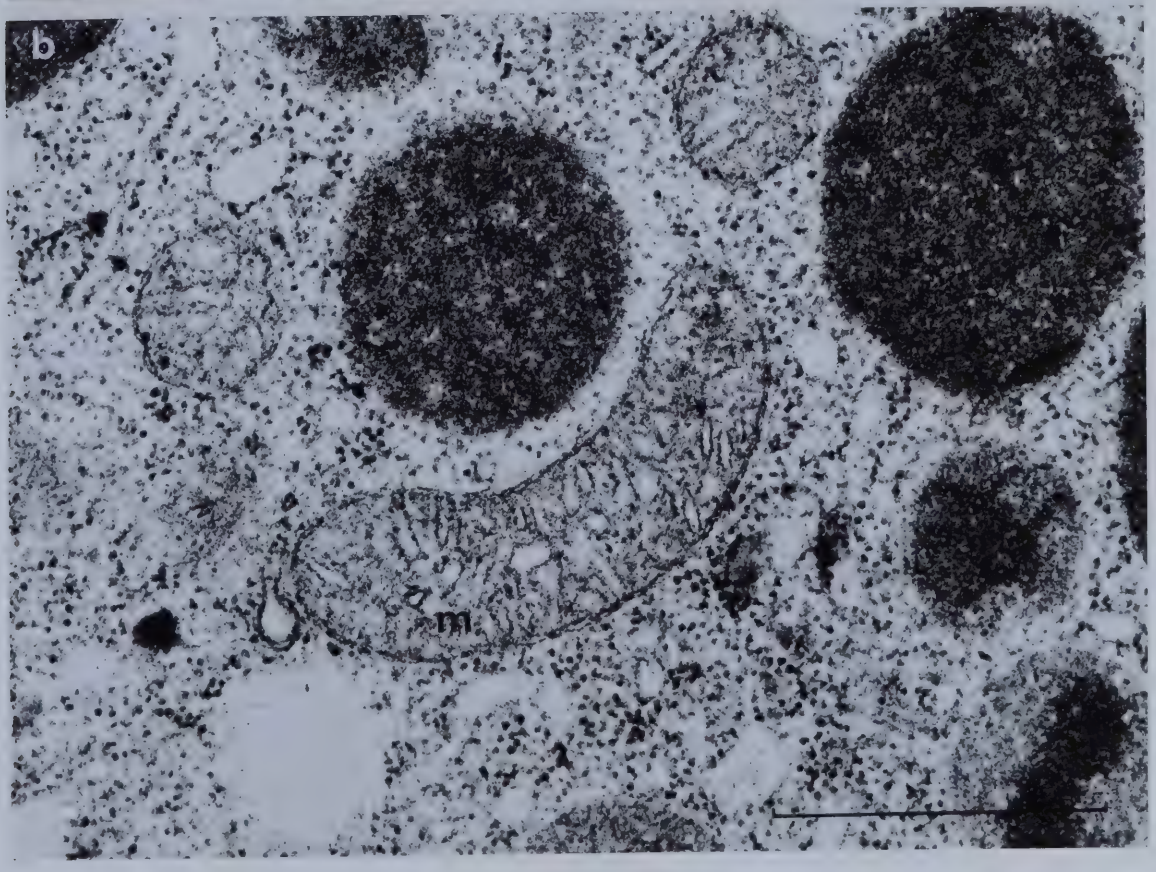
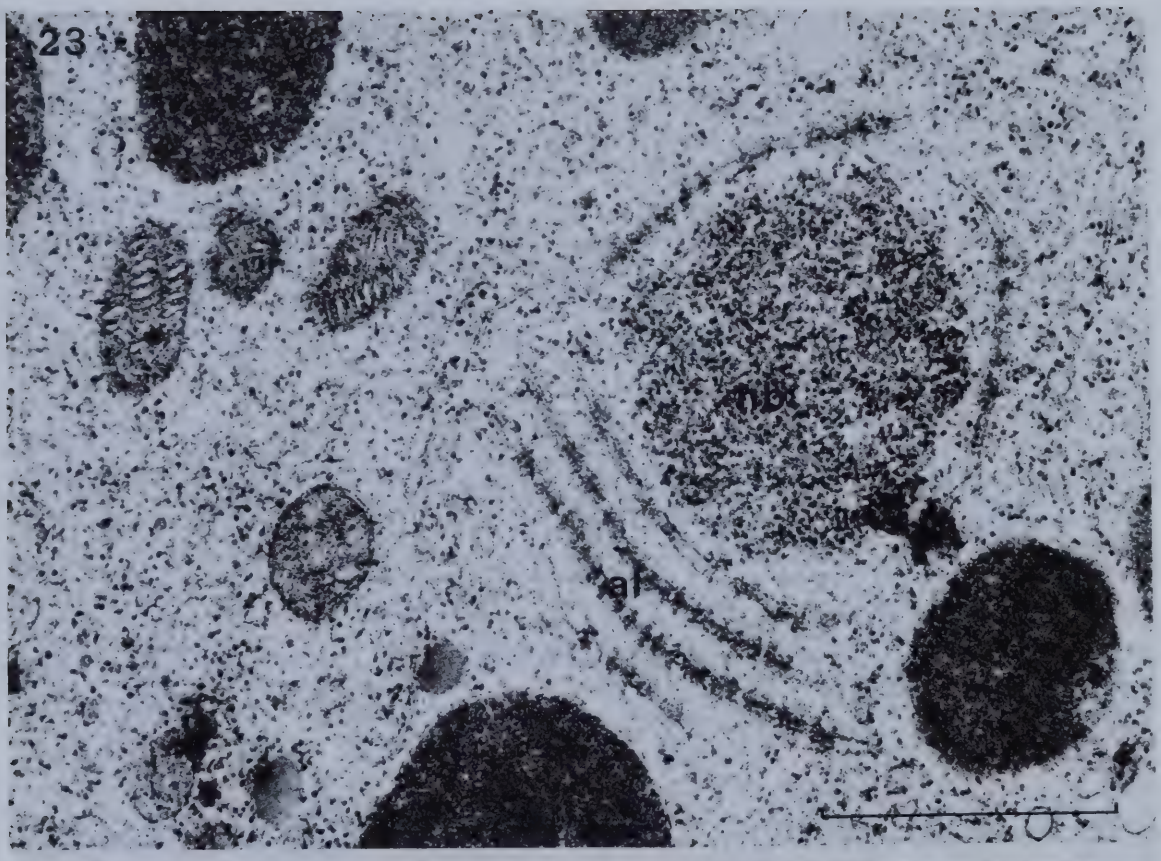

b
m

Figure 23. Annulate lamellae appear in the cytoplasm of the primary oocyte after the onset of vitellogenesis. Heavy bodies are associated with the annulate lamellae. al, annulate lamellae; hb, heavy bodies.

Figure 24. Tangential section of the nuclear membrane and the annulate lamellae which originate here. al, annulate lamellae; cyt, cytoplasm; n, nucleus.


23
nb
al

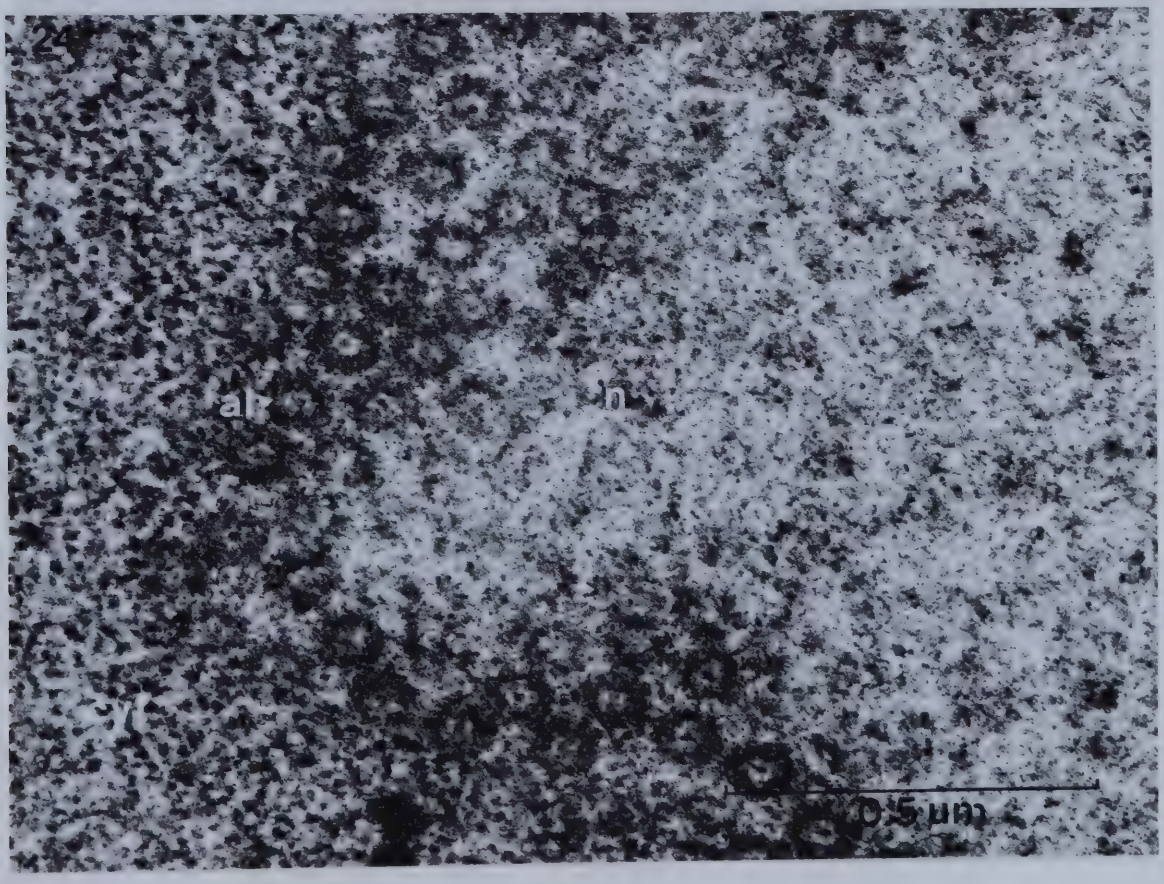
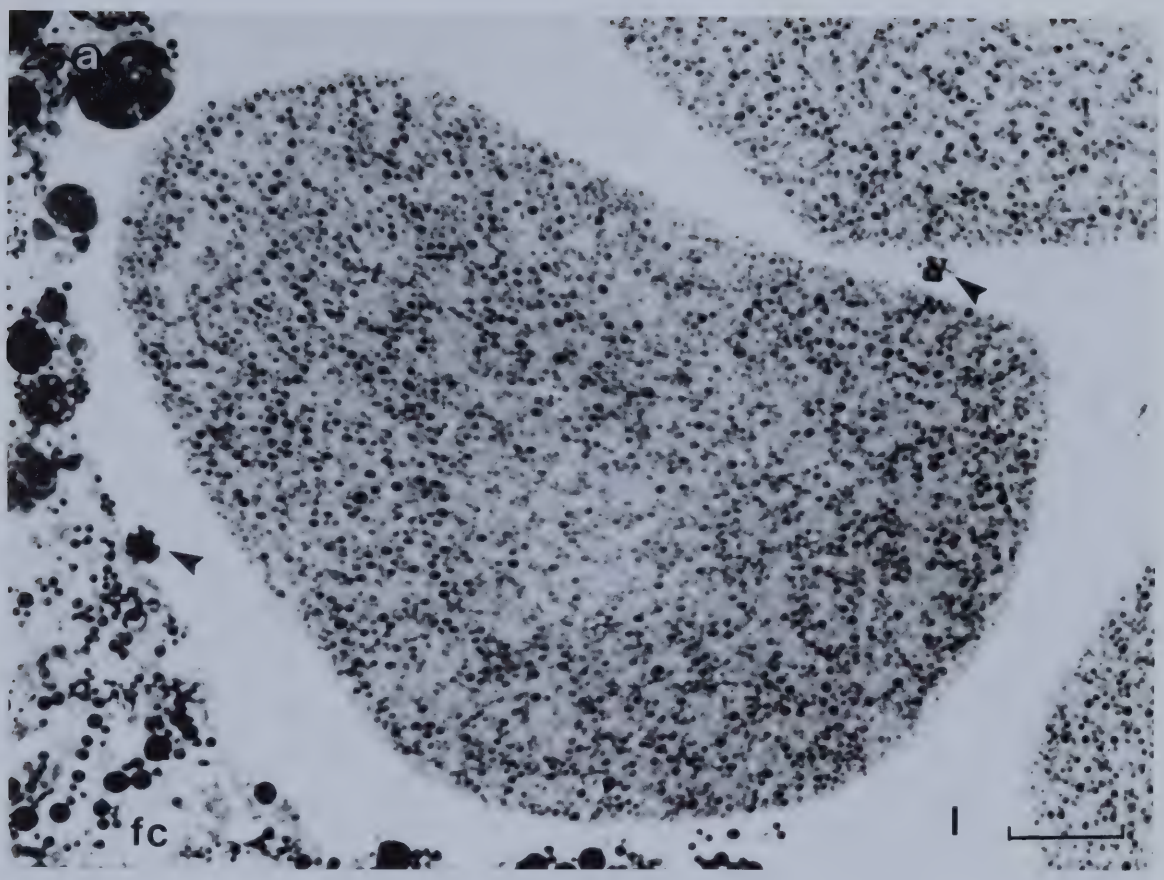

24
al
n
0.5 µm

Figure 25. Light micrographs of ovarian tubules. a) Oocyte in lumen of ovarian tubule. Note that the oocyte is not associated with the follicle cells. The pigment cells are elevated into the jelly coat (arrows). b) Cross section of an ovarian tubule showing oogonia and oocytes associated with the follicle cells. Primary oocytes with their pigments cells (arrows) elevated into the jelly coat are also shown. fc, follicle cells; l, lumen; o, oocyte; oo, oogonium.


a
fc
l

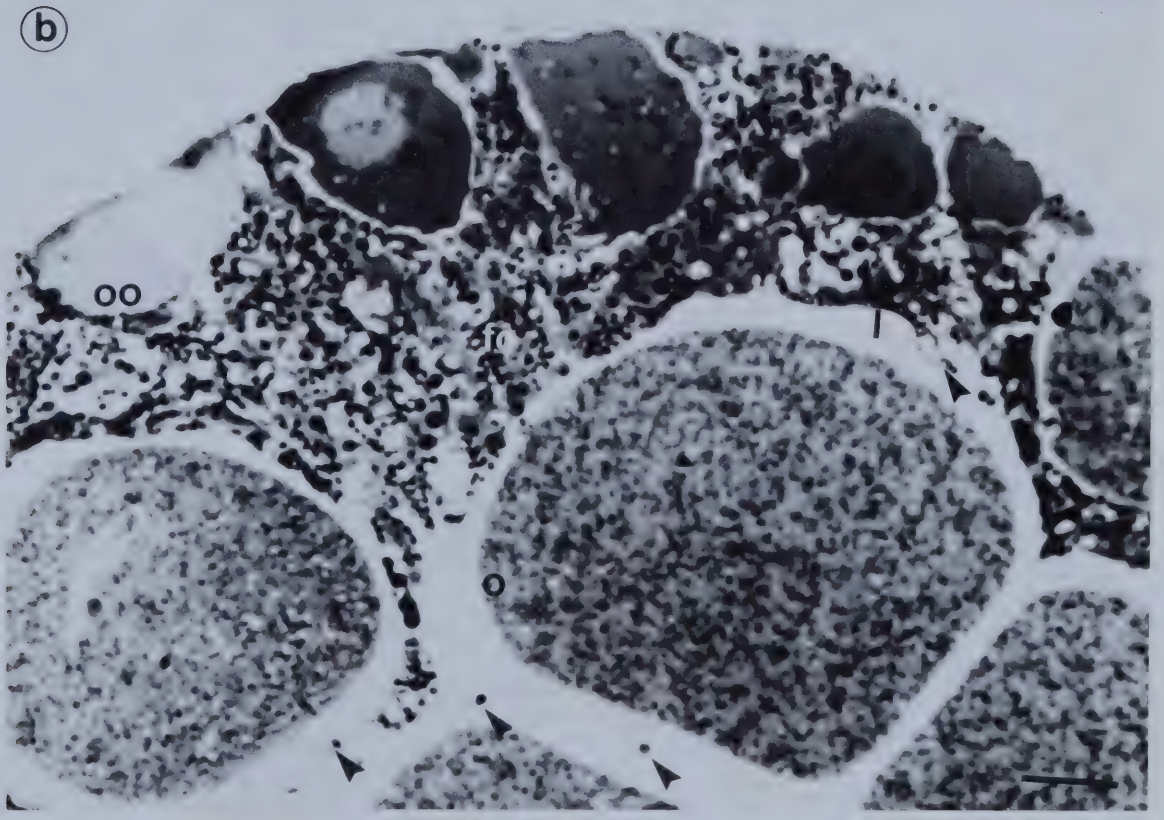

b
oo
o

Figure 26. Different types of cortical granules of *Dendraster excentricus*. a) Type 1. b) Type 2. c) Type 3. d) Type 4. e) Type 5. f) Type 6.

26

a

b

c

d

e

f

Figure 27. Electronmicrographs of cortical granules. a) Types 1 and 5. b) Types 2 and 3. c) Types 1 and 4. d) Types 1 and 6. It is possible that types 1-3 represent different sections from the same type.

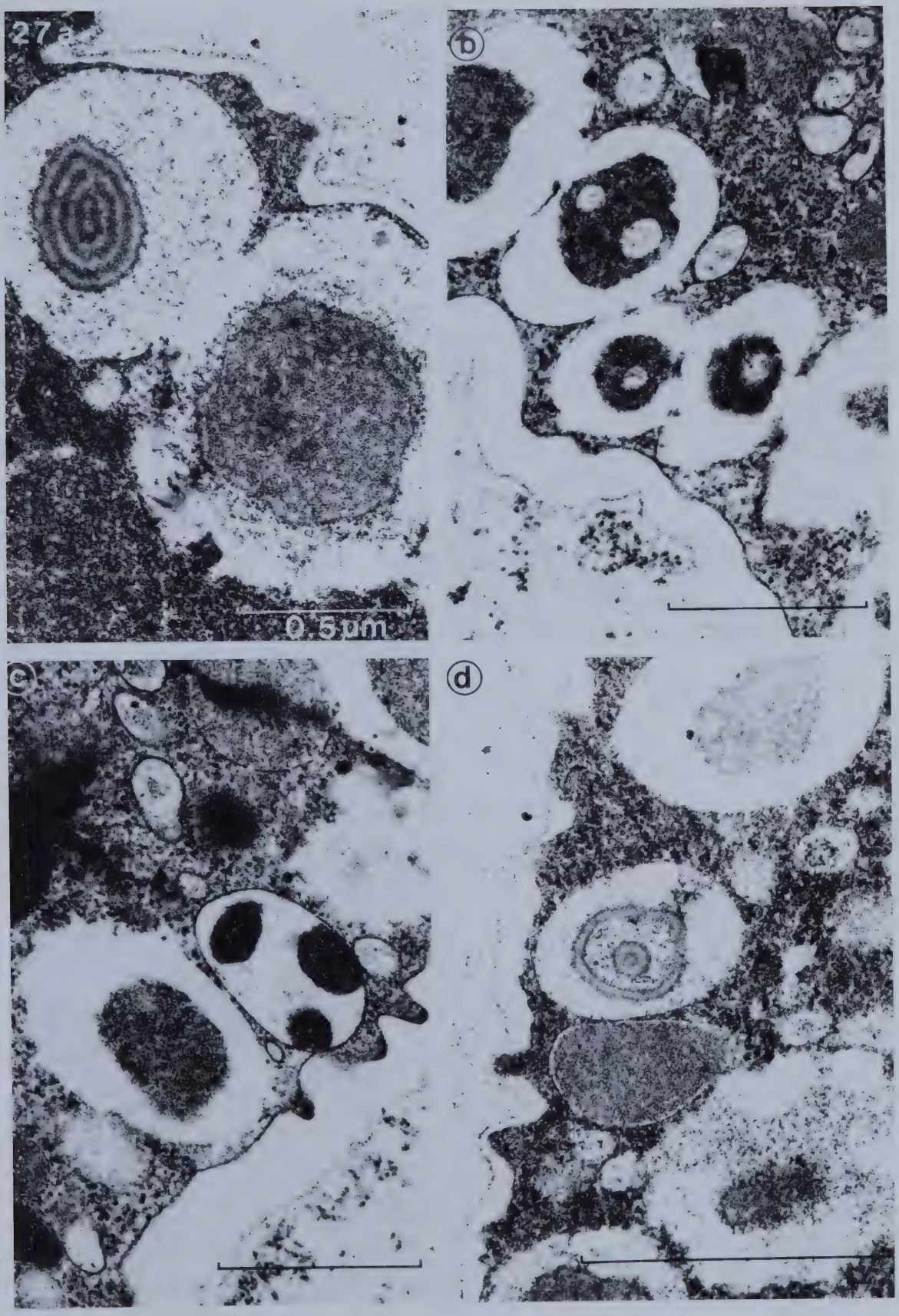
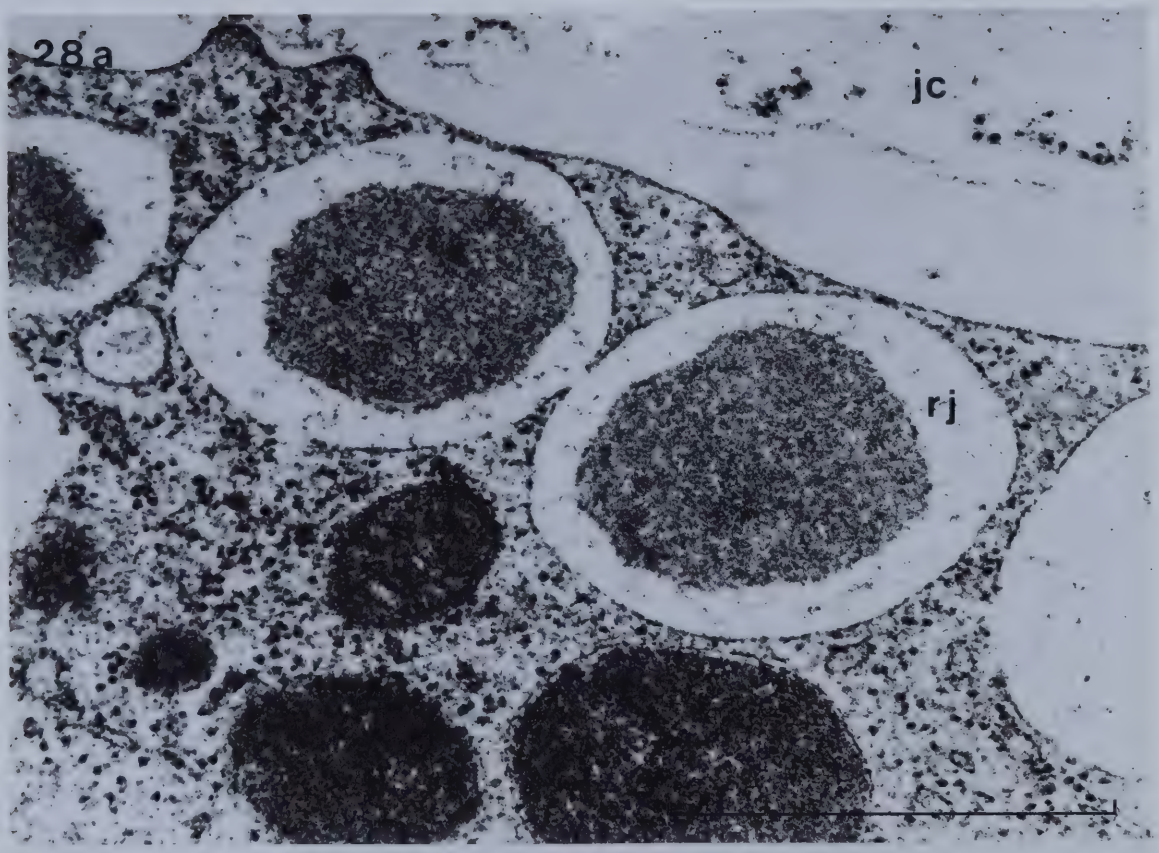

27
0.5 µm
b
c
d

Figure 28. Cortical granules a) less electron dense area resembles the jelly coat in its stain retention. b) Types 1 and 2. jc, jelly coat; rj, resembles jelly coat.


28a
jc
rj

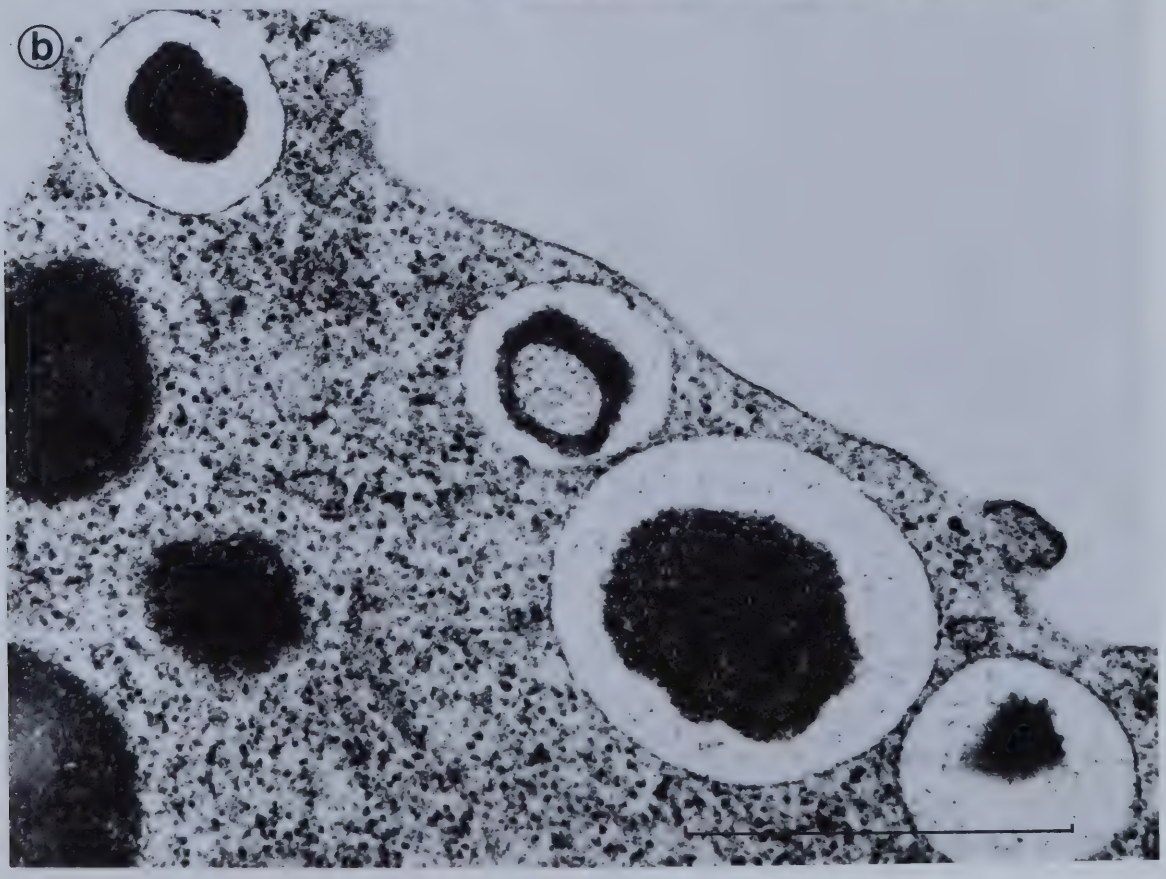
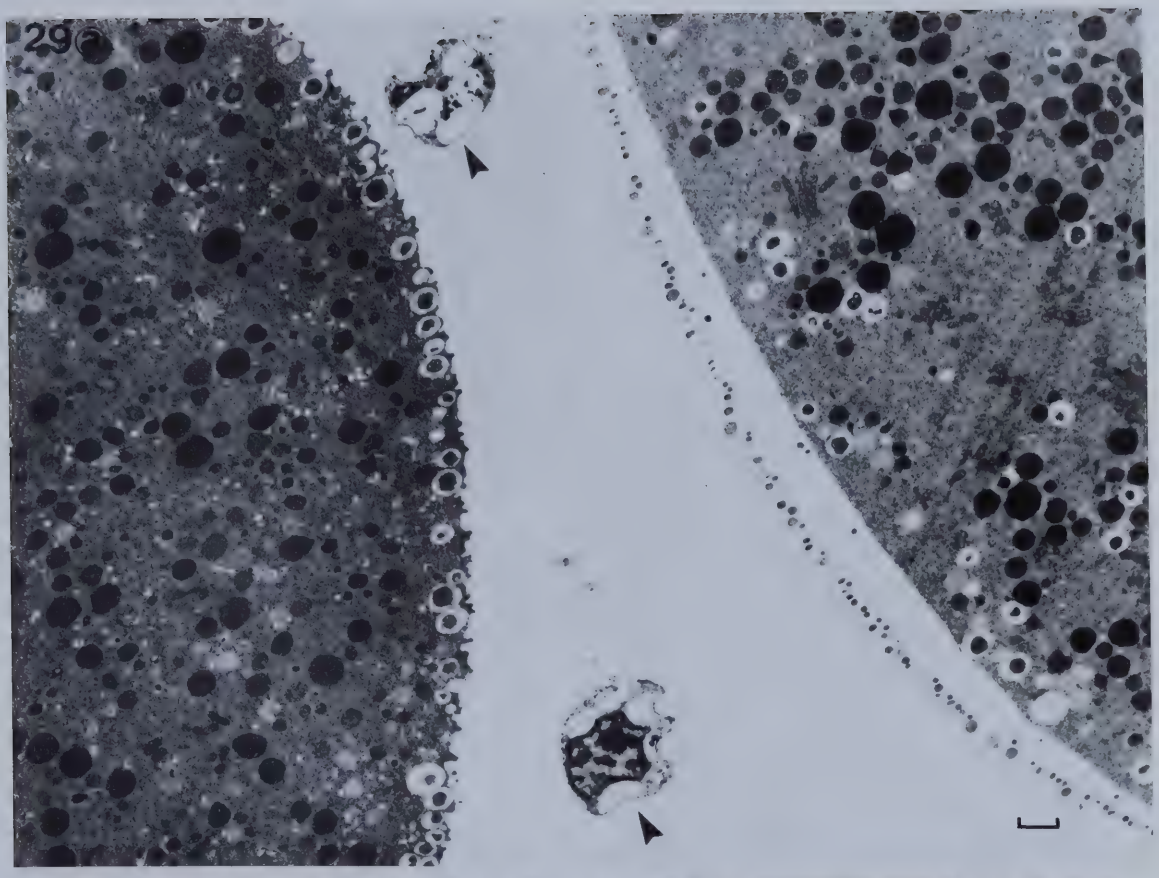

b

Figure 29. The red pigment cell. a) A mature oocyte (left) identifiable by its cortical granules aligned in the cortex, and the pigment cell (arrow) which has been elevated into the jelly coat. The oocyte on the right is a primary oocyte. b) Pigment cell (arrow) situated amongst the follicle cells.


29a

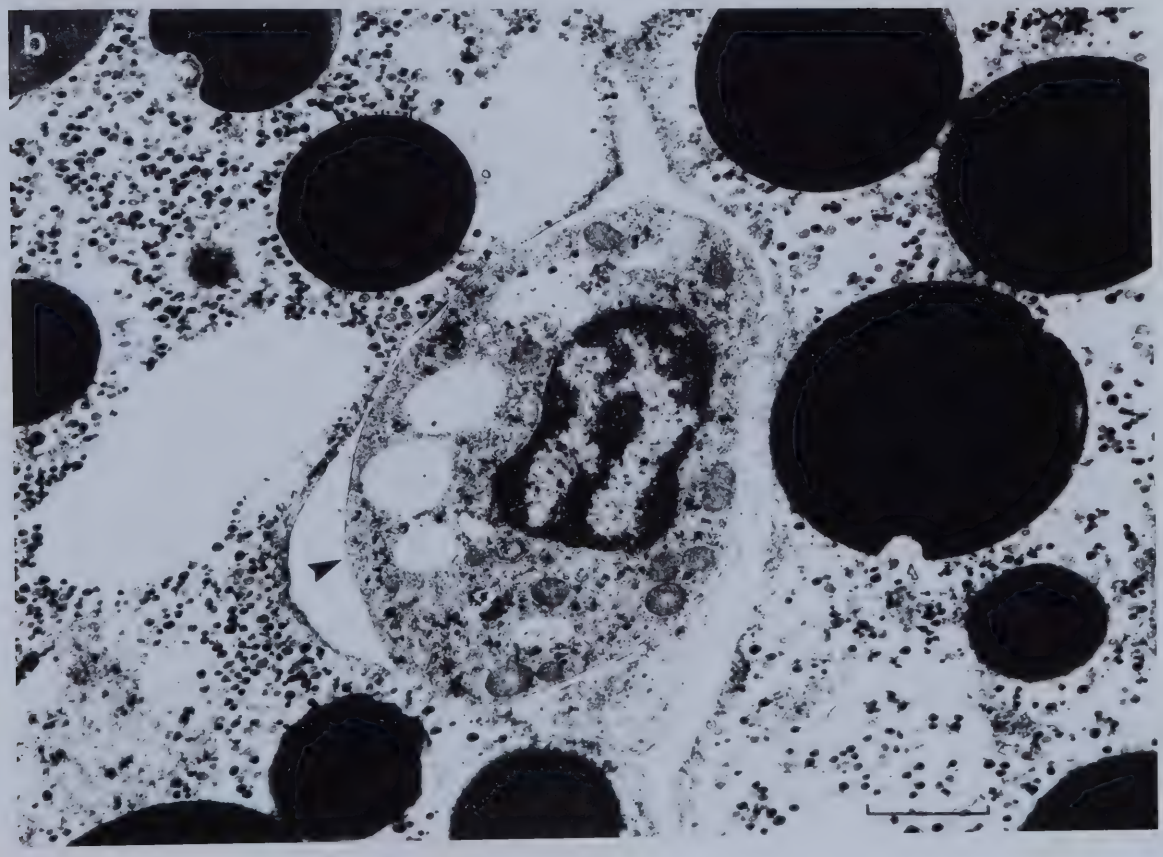

b

Figure 30. Pigment cells in association with the oolemma. a) Note the arrangement of the chromatin (arrows). b) Densely staining bodies (arrow) may be nucleoli.

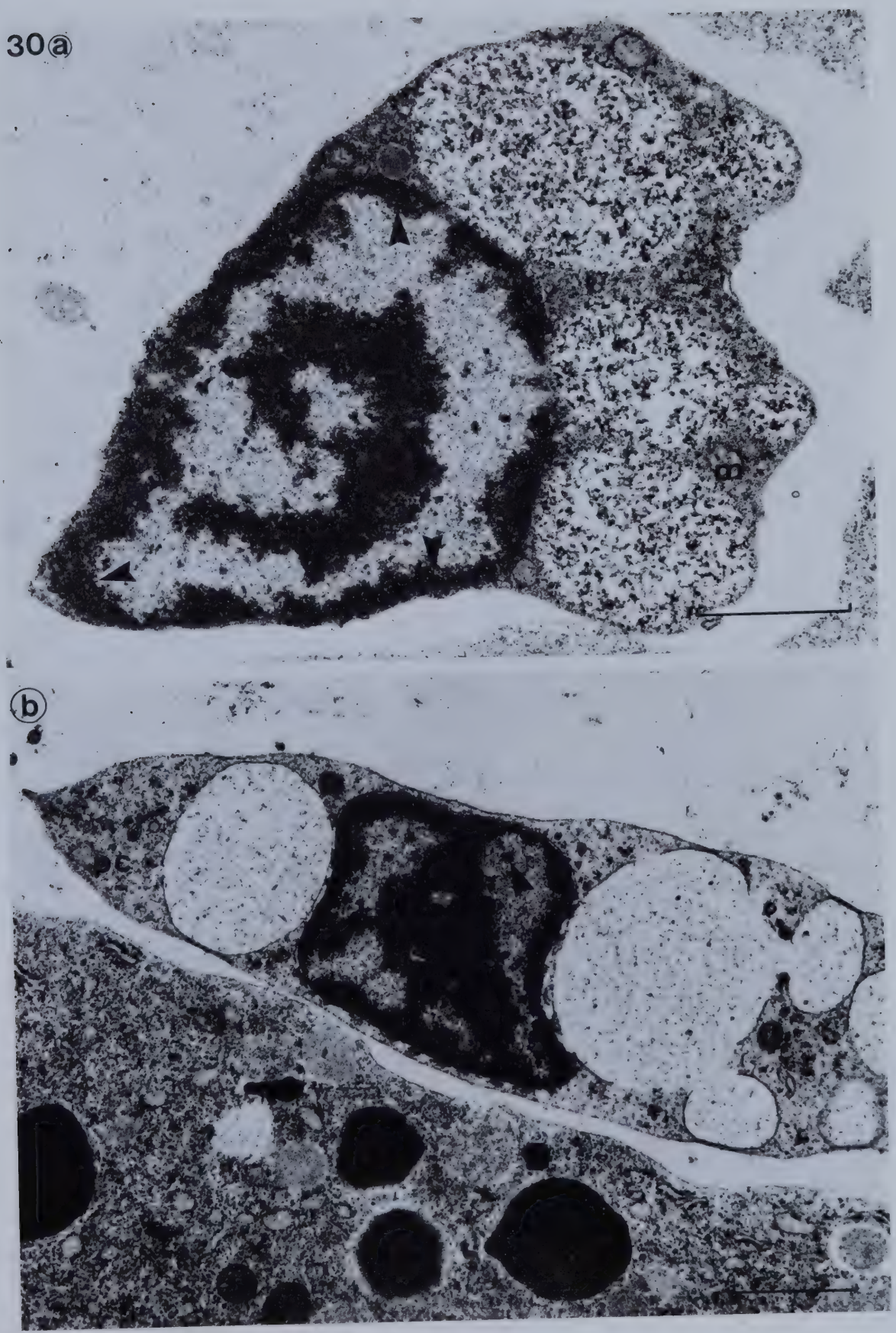
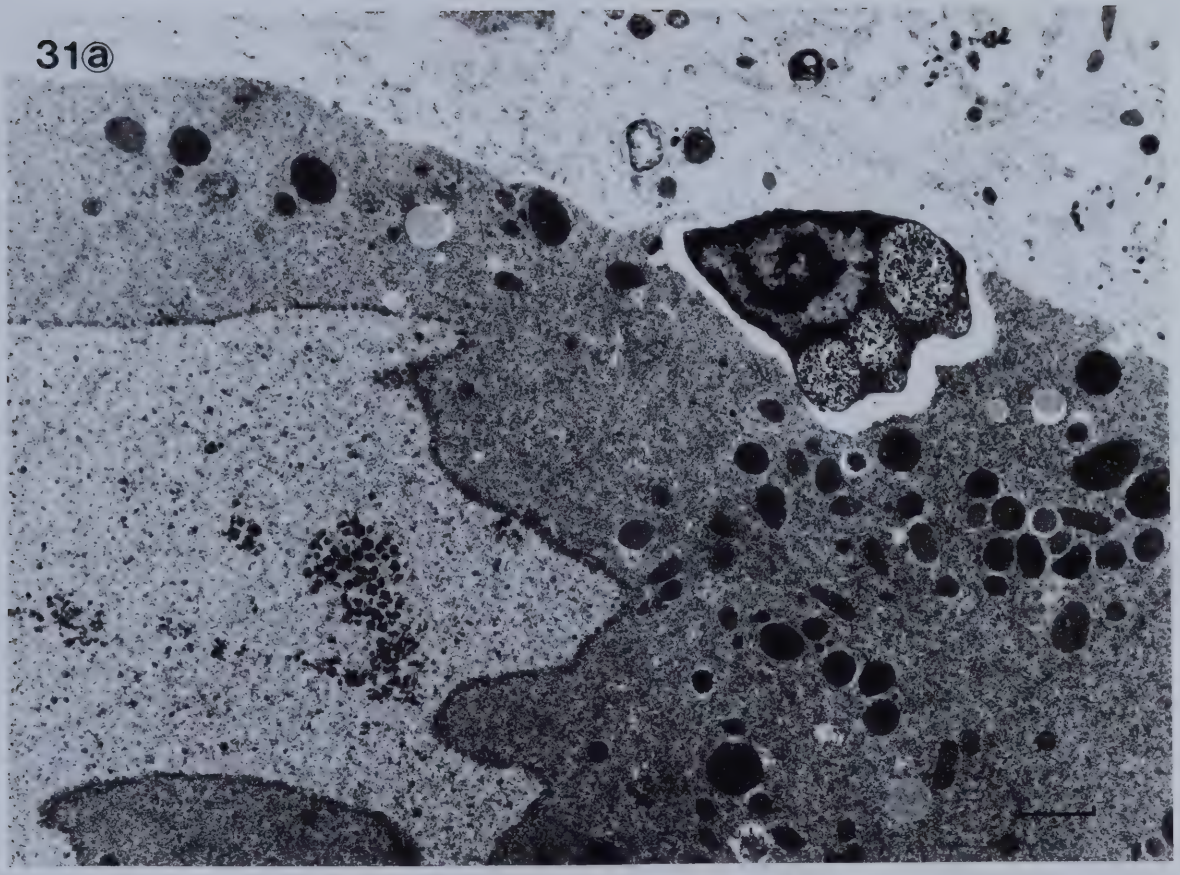

30@
a
b

Figure 31. Pigment cells in differing stages of being pocketed along the oolemma. a) The pigment cell in this micrograph is fully pocketed. b) A depression is forming in the oolemma which will later pocket the pigment cell. Note the manner in which the follicular extensions surround the oocyte and its pocketing pigment cell.


31ⓐ

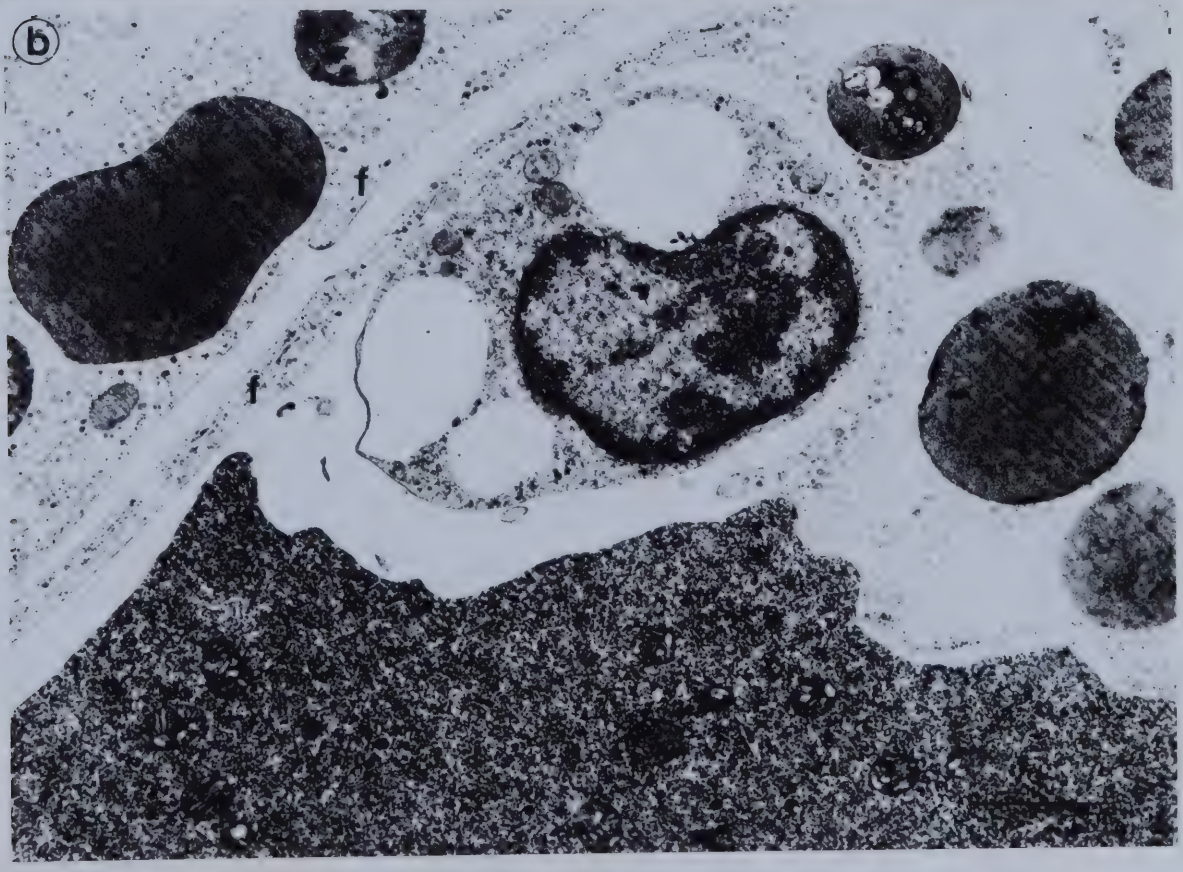

ⓑ
f
f

Figure 32. Light micrographs of the primary oocyte. a) pocketed pigment cells (arrows) surrounded by follicular extensions. Note distinct nucleolus. b) Enlarged micrograph showing pocketed pigment cells of primary oocyte. Follicular extensions are evident. Note dispersed chromatin in nucleus of oocyte.

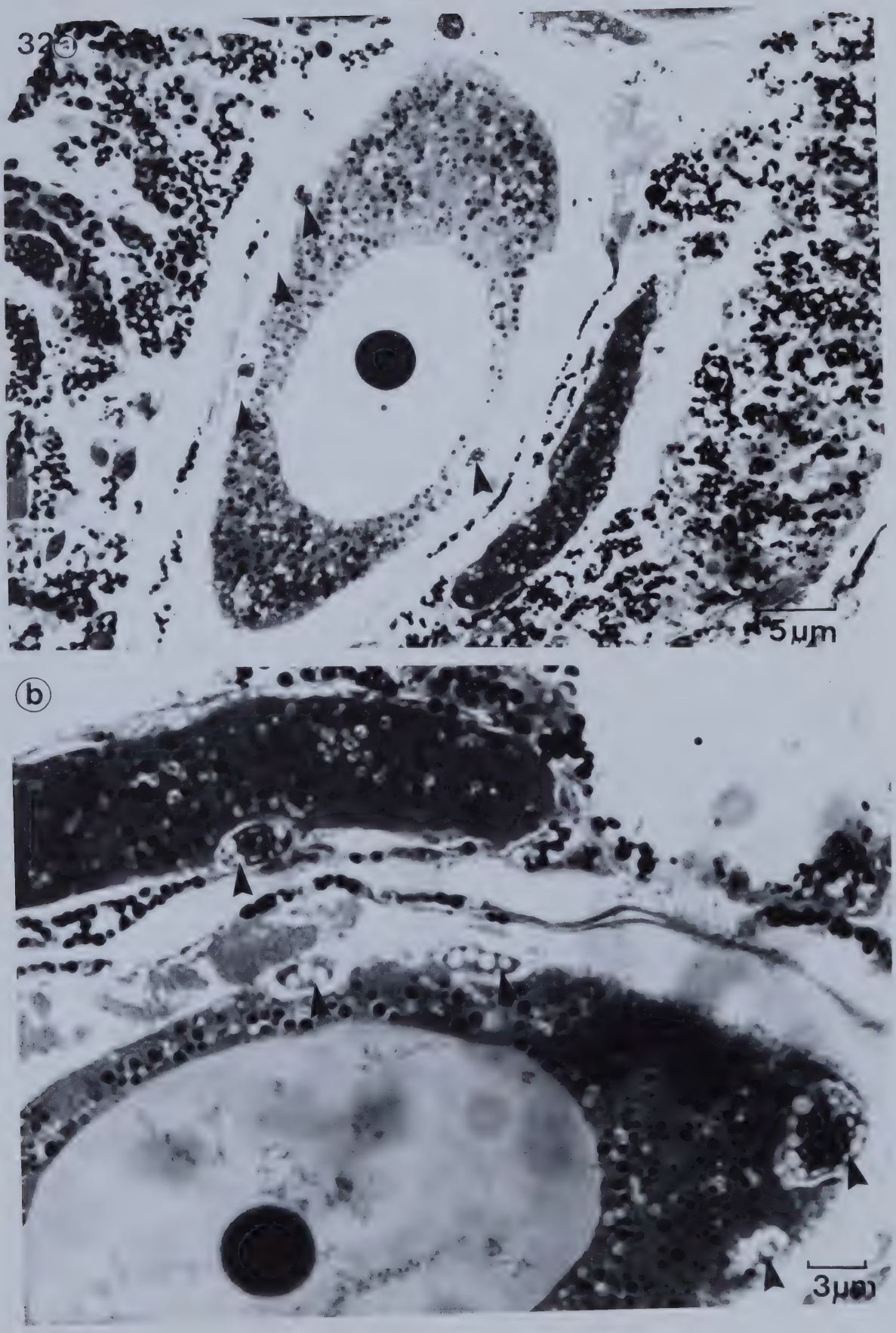

32
5µm
b
3µm

Figure 33. Follicle cells. a) Note that the granules of the cytoplasm as well as the ribosomes are sparse. b) Electron dense bodies of similar appearance and stain retention are found in both the follicle cells and the primary oocyte. fc, follicle cell; o, primary oocyte.

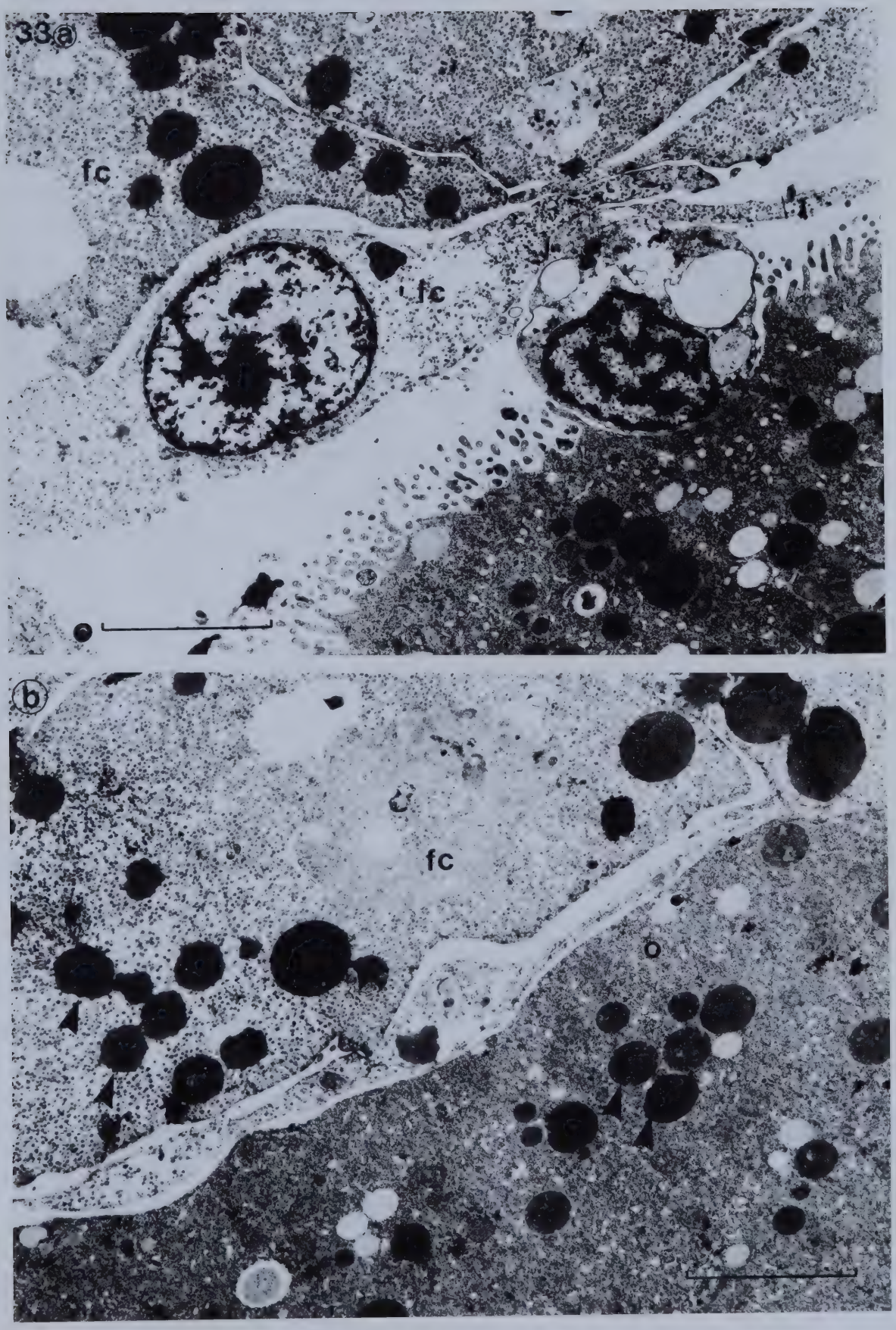
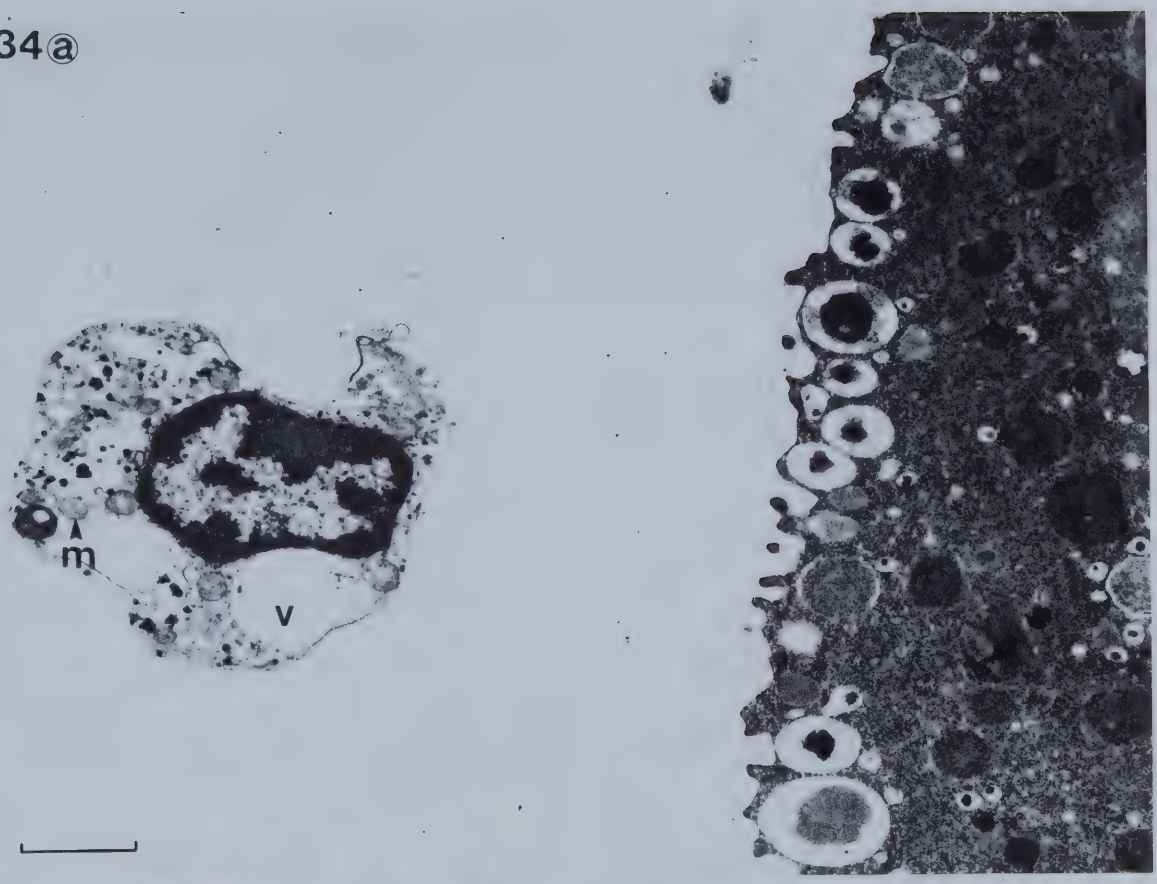
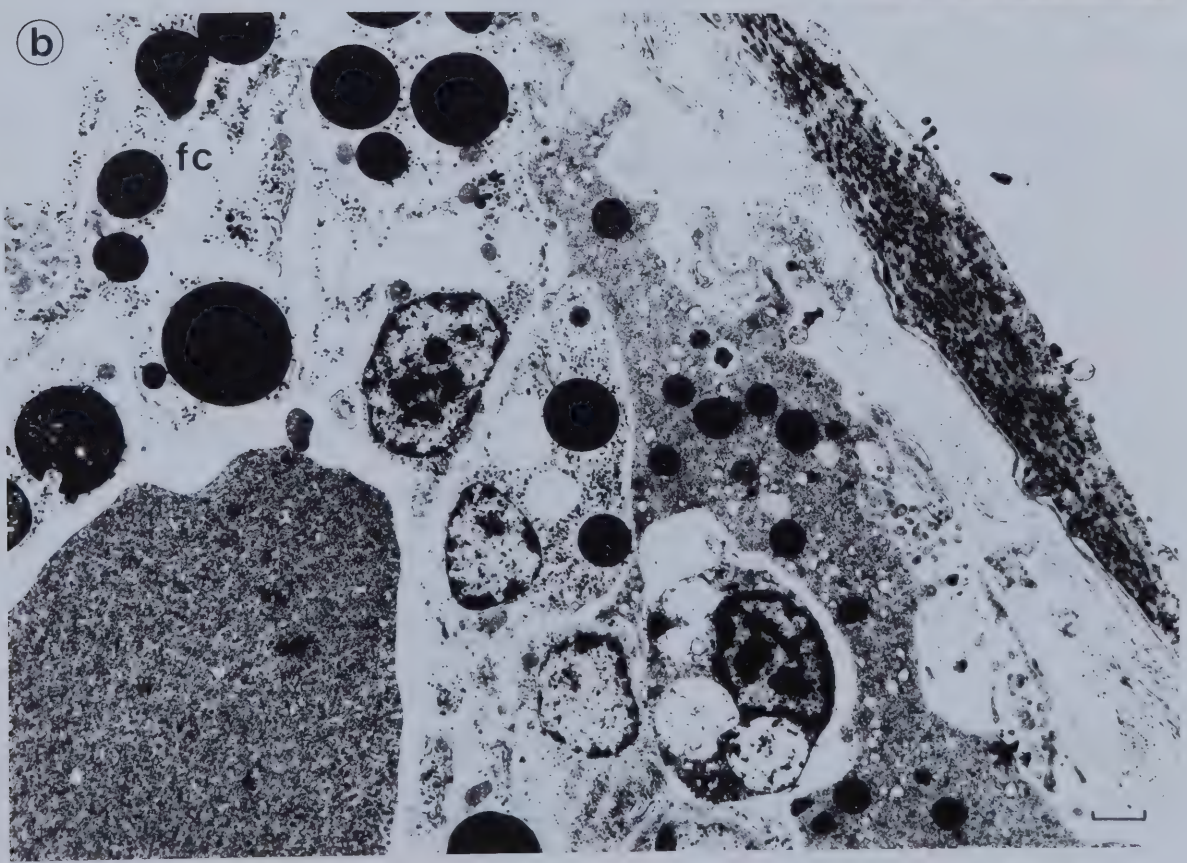

33a
fc
fc
b
fc
o

Figure 34a. Mature oocyte with pigment cell elevated into the jelly coat. The cytoplasm of the pigment cell appears to be degenerating. The ribosomes are no longer distinct and the mitochondria are not as well defined as they were previously. m, mitochondria; v, pigment vacuole.

b. Follicle cells developing at the germinal epithelium. Note the number and arrangement. fc, follicle cell.

34@



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